QUALITY CONTROL OF MASS-REARED LEPIDOPTERA USING THE FALL ARMYWORM, *Spodoptera frugiperda* (J.E. Smith), AS A MODEL

By

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

QUALITY CONTROL OF MASS-REARED LEPIDOPTERA USING THE FALL ARMYWORM, Spodoptera frugiperda (J.E. Smith), AS A MODEL

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A series of tests and observations on developmental, morphological, and behavioral traits was developed for evaluating the quality of fall armyworms, Spodoptera frugiperda (J.E. Smith), reared on artificial diet in communal containers, and for establishing a model quality control program for mass-reared lepidopterans. Black mold grew on the diet surface in 35% of the containers and was especially prevalent on Mondays, Wednesdays, and Fridays, the busiest days for insectary employees. The incidence of mold was correlated with reduced pupal abdominal rotation and buoyancy, increased pupal mortality, and unsuccessful adult eclosion. Process control charts were shown to be sensitive tools for identifying changes in insects during colonization. Average pupal weights showed some

out-of-control points caused by high larval densities. Downward trends were noted in sample averages for weight and rotation but sample ranges were within limits of normal variability. Pupal mortality averaged 1.6% and was significantly higher when mold was present. Wing deformities occurred in 4.3% and 5.2% of males and females, respectively. Wingbeat frequency (WBF) for males averaged 44.7 cycles per second and remained within limits; moths from heavier pupae had reduced WBF. About half of the laboratory males demonstrated anemotactic flight in response to pheromone-producing females, compared to 83% for wild males. Only 73% of the laboratory males arrived at the source in an average of 65 sec while all of the wild males arrived in an average of 39 sec. Adult diet and pupal radiation treatment altered mating behavior and success. Untreated laboratory males attempted to clasp a female once every 15 sec whereas males from a group of irradiated pupae averaged once in 82 sec, and unfed males pursued females for an average of 120 sec but never attempted copulation. Results were incorporated into a model quality control program based on an integrated facility designed to ensure efficient production in a sanitary environment, procedures that allow natural behavior of larvae and adults, a battery of laboratory quality control tests, process control charting, and a system that feeds data from tests directly back into the production program.

INTRODUCTION

Quality Control of Colonized Insects: A Historical Perspective

Quality control has been practiced as long as man has maintained insect colonies. For many years, quality insects were considered those that were merely alive. However, during the early 1960's, published accounts demonstrated that sublethal changes in colonized insects severely reduced potential effectiveness of pest control strategies. For example, Weidhaas et al. (1962) reported unsuccessful attempts to introduce sterility into a wild population of Anopheles quadrimaculatus Say using irradiated laboratory males. Investigating possible causes for this failure, Dame et al. (1964) showed that the problem resulted from an inability of the males to disperse in the wild and seek out mates. In 1962 sterile laboratory flies were released in the southwestern United States to control the screwworm, Cochliomyia hominivorax (Coquerel), which caused significant economic losses to the livestock industry. A precipitous drop in screwworm infestations occurred within one year and the program was hailed as a milestone in pest control. However, within a decade the release of laboratory flies in numbers five times greater than in 1962, proved ineffective

in controlling the screwworm. Lack of control was attributed to laboratory insects of poor quality. Alley and Hightower (1966) reported that newly colonized screwworms mated less frequently than laboratory-adapted strains and mating frequency for small screwworm flies was significantly lower than for larger flies no matter how long they remained in culture. Spates and Hightower (1967) found that males from recently colonized strains of screwworms did not harass laboratory females as vigorously as did males from older strains.

In 1966, C.N. Smith edited a book entitled "Insect Colonization and Mass Production" that described procedures and problems associated with rearing over 40 species of insects, mites, and ticks. Most authors stressed the importance of the laboratory environment on production output and insect quality, although the latter term was infrequently used. Emphasis was placed on preventing contamination of diet and insects, especially by disease organisms. Cleanliness of the rearing facility and its equipment, and sanitary techniques were discussed by Cowan, Gast and Davich, Henneberry and Kishaba, and others. The importance of isolating wild colonizers to ensure disease-free stock prior to introduction into the laboratory was recommended by Harein and Soderstrom. Difficulty in establishing wild insects in the laboratory was a common problem and those authors that addressed the issue concluded that a large colonizing population was the best means to

ensure survivors. Gahan reported difficulty getting wild mosquitos to mate in capitivity and suggested a forced mating ritual for fertilization. De Meillon and Thomas recommended selecting for variability in a laboratory colony of mosquitos by using only those larvae and adults that showed vitality.

Some authors discussed measuring and evaluating insect quality. Lumsden and Saunders listed several parameters for monitoring colonies of tsetse flies. They included rate of population increase, longevity and mating frequency of individual females, number of pupae per female, length of larval period, and pupal weight. Baumhover et al. described a quality control program for irradiated screwworm flies that included measurements taken on viability of treated pupae, sterility and longevity of adults, mortality resulting from storage and shipment to the release site, sexual aggressiveness, and field tests of competitiveness. Gahan described differences in behavior between wild and laboratory mosquitos and he cautioned against predicting field behavior from studies conducted in the laboratory. Lumsden and Saunders addressed the same problem with tsetse fly and indicated that frequent gene reinforcement from wild populations may be required to improve field competitiveness of the laboratory colony. Smith's book illustrated the diversity of species being reared and identified basic problems common to the production of most of them.

During the same year, a conference of the International Atomic Energy Agency (IAEA) updated insect rearing information to improve sterile release technology. R.T. Gast (1968) stated the objective of mass rearing to be the production of an acceptable insect at the lowest possible cost. He defined an acceptable insect as one that was inevitably different from wild, but one that could perform in its intended role of pest suppression. He stressed the development of standards of acceptability. Once an acceptable insect was being reared, emphasis should be placed on reducing production costs. Gast also discussed the significance of selection and microbial contamination in reducing insect quality. Recommendations of the IAEA panel included designing rearing facilities and equipment to reduce the potential for contamination, defining symptoms of nutrient deficiencies, and establishing quality control criteria to optimize insect performance in field releases.

In 1970, a similar panel discussed the application of induced sterility for the control of Lepidopterous pests (IAEA 1971). It reported that mass production "represents one of the most difficult obstacles to immediate field testing of the sterility principle for a great many of these pests", with disease and nutrition being of specific concern. To help alleviate these problems, they recommended compiling and disseminating information about the design and construction of rearing facilities including specifications for equipment and building materials such as wall and floor

finishes. During these and other IAEA proceedings, discussions centered on the deterioration of insect quality (e.g. dispersal, orientation to environmental stimuli, and mating behavior) resulting from exposure of the insects to sterilizing radiation.

In 1971, a symposium sponsored by the International Organization for Biological Control (IOBC), "Implications of Permanent Insect Production" was held in Rome. Two timely papers resulted from the conference. The first, by E.F. Boller (1972), discussed changes in behavior during laboratory colonization of wild insects. These changes, based on genetic or environmental factors, were especially apparent during generations one to six, after which colonies became stabilized, albeit different from their wild conspecifics. Boller suggested modifying the traditional concept of mass-production--i.e., the number of fertile insects per dollar--to the number of insects required to achieve specified objectives. Steps to help ensure production of quality insects included strategies for maintaining genetic variabiltiy and monitoring insects for quality control using various behavioral tests in the laboratory and field.

The second paper dealt with the genetic changes that occur during colonization. Mackauer (1972) stated that preservation of essential attributes (adaptability, competitiveness, and mobility) during colonization was dependent on, among other things, the size and origin of the

founder colony. It must be large enough to assure genetic flexibility and thus survival under laboratory conditions. He suggested that large founder populations are less susceptible to random genetic changes (i.e. drift) and have greater genetic diversity, resulting in a laboratory strain that is similar to wild insects.

At that time, examples of poor performance by laboratory insects showed that success of programs such as sterile insect technique (SIT) and release of beneficial control agents was intimately related to colonization and production processes. To investigate this relationship, a workshop in 1974 dealt with the genetics of insect behavior and its relation to insect production and quality control. The workshop was organized by D.L. Chambers, W. Klassen, L.E. LaChance, I.C. McDonald, R.L. Ridgway, and D.E. Weidhaas. Participants identified objectives for future activities based on improving poor performance. These included developing a systematic approach to measuring quality in the laboratory and field, and incorporating the results back into the production system to make required changes; monitoring founders as soon after collection as possible for identification of the precise changes that occur during colonization; using data processing to simplify quality control efforts; eliminating disease and identifying the effects of sublethal concentrations of pathogens; determining whether changes in behavior are induced by genetic or non-genetic factors; establishing standards,

especially with field insects; and identifying the role of nutrition in fitness. The conference also recommended elevating the study of insect quality to a higher priority, organizing research groups, workshops, and task forces accordingly, and instituting educational courses to familiarize production personnel with relevant principles of ecological, quantitative, and population genetics. This workshop provided the comprehensive and integrated base upon which future quality control philosophy and application were developed. Further, it educated many scientists and helped coordinate their activities in the field.

In 1975, D.L. Chambers outlined basic concepts of quality control and defined important terms. He defined quality as the degree of excellence in some trait relative to a standard, usually the wild insect, or more simply, fitness for use. Problems with measuring traits of wild insects, however, sometimes made the use of such standards infeasible. Therefore, comparison with an internal standard or the untreated laboratory insect was recommended. While its use was not necessarily a measure of fitness in the field, the internal standard did provide a reference value for detecting changes in stock quality. Chambers stressed the importance of regular measurements of performance traits using a variety of parameters, and he stated that success of the release program was an inadequate indicator of quality because it did not necessarily ensure continued production of quality insects. Further, the use of only one or two

traits to estimate field performance may be inaccurate and misleading. Rather, he suggested a series of laboratory and field tests to evaluate overall quality, or how well the insect performs its function in the field. Those tests that measure adaptedness (physiological functions, genetic divergence), motility (capability and propensity to move), and reproductive success are most important.

A symposium entitled "Solving Insectary Production Problems" was held in 1975 as part of the Eastern Branch meetings at the national conference of the Entomological Society of America. Two papers are of special interest. First, Huettel (1976) summarized some of the tests and techniques used to monitor quality of colonized insects. They will be discussed together with those suggested by Chambers (1975). Changes in gene frequencies of colonized populations were monitored by allozyme electrophoresis which detects the relative movement of charged enzyme molecules in an electric field. Life history measurements were made directly on developmental rates, weights or size, fecundity, fertility, longevity, and mortality. Physiological processes were monitored by exposing samples of insects to stressors such as extreme temperatures, relative humidity (RH), or insecticides, and by determining uptake rates of oxygen or production rates of carbon dioxide. Testing the insects' ability to locate food sources and to avoid predators measured survival potential. Periodicity of activity was determined by actographic techniques that used

various types of physical sensors to detect movement of caged insects. Rhythmicity was also established by analysing carbon dioxide output or recording sounds produced by the insects. Motility was monitored by measuring flight ability and propensity. Flight behavior was studied using a flight mill which consisted of a horizontal rotor attached to a perpendicular support in such a way that it was free to rotate. An insect was tethered with quick-setting glue at one end of the rotor and an opaque flag was attached to the other. As the insect "flew" in circles, the flag passed between a photocell and a light source, opened an electrical circuit, and produced a mark on a strip chart recorder. Information was measured on frequency, velocity, endurance, and distance flown. Wingbeat frequencies were determined stroboscopically or by acoustical analysis. Flight propensity was measured by startling insects with a stimulus that would normally cause flight, such as light impinging on dark-adapted adults. Orientation to visual and chemical stimuli was also monitored. The electroretinogram (ERG) technique, using minute electrodes implanted in the eye, recorded stimulation of optic fibers. Thus, spectral sensitivities and threshold response for monochromatic light of varying intensities were measured. Preference tests using various colors were used to evaluate behavioral responses to visual stimuli. Olfactometers and flight tunnels were useful in monitoring the response of insects to chemical stimuli, such as host plant attractants and

pheromones. The qualitative, quantitative, and periodic release of pheromone production was also monitored. Reproductive success was measured by the ratio test where various proportions of treated (e.g. irradiated) and untreated males were caged together with untreated females. Percentage fertility indicated the relative degree of competitiveness of the treated insect.

The second paper of interest at the 1975 symposium discussed the potential of genetic improvement of mass reared insects (Hoy 1976). Successes in silkworm and honeybee production were cited as examples of progress that has been made. Hoy stated that before genetic gains are possible, effective rearing methods must be developed, desirable attributes must be clearly defined, and adequate genetic variability must exist. She concluded that genetic aspects of the laboratory colony must be considered during development of the production program and that genetic quality is aided by colonizing representative samples from the field, maintaining colonies under natural conditions, and preventing the intense genetic selection that usually occurs in early generations of laboratory populations.

In 1977, two major publications were responsible for the general acceptance of quality control concepts and for their implementation into mass-production programs. First, an article entitled "Quality Control in Mass Rearing", by Chambers (1977), appeared in the Annual Review of Entomology. The mechanisms of change in laboratory insects

and the need for quality control were summarized so that entomologists, even those unfamiliar with insect production systems, could understand the scope and importance of the problem. As such, it may have been one of the most important milestones in the development of insect quality control.

In addition, a handbook on quality control and monitoring techniques was published for individuals involved in the production of fruit flies. The book, "Quality Control: An Idea Book for Fruit Fly Workers", was edited by Boller and Chambers (1977). In it, the concept of industrial control charting was introduced as a graphical means to identify changes in the quality of insects. With this aid, significant deviations in traits measured over time could be detected and used to help identify and solve problems in the production system. Many tests for monitoring essential traits in fruit flies were presented. Also, Leppla et al. (1977) discussed evaluating insect adaptedness using life tables and other developmental data. They emphasized the need for a thorough understanding of all rearing operations and the effects they have on all developmental stages. The idea book simplified the application of quality control and provided the basis for the development of standardized programs.

In 1979, the IOBC and other national and international organizations sponsored an International Course on Quality Control in Ceratitis capitata (Wiedemann) in Spain. It was

organized by E.F. Boller, C.O. Calkins, D.L. Chambers, and N.C. Leppla. In this training program, the RAPID Quality Control System was introduced, evaluated, and accepted as international test and evaluation procedures for the Mediterranean fruit fly. RAPID was designed for quick and simple measurement of key insect traits in laboratory and field tests. They measured a developmental characteristic, motility traits, and sexual activity. Pupal size was used as an indicator of stressful conditions within the production system and as a correlate of flight potential. A machine divided insects into several categories according to diameter. Puparia were placed between two sloping steel cylinders with the distance between cylinders increasing slightly from top to bottom. Smaller diameter insects fell through to collection boxes near the top and larger ones at the bottom.

The RAPID system tested both flight ability and propensity. A test of the first trait measured the ability of adults to fly from a container that consisted of a cardboard tube coated on the inside with paint to reduce friction and prevent flies from walking out. The tube was placed on top of a petri dish containing a sample of puparia. After emergence, able flies left the tube and remaining insects were classified according to eclosion success, wing deformity, and pupal mortality. Flight propensity was measured using a startle test in which dark-adapted flies were briefly exposed to an overhead

light. The number of insects arriving at the light indicated their propensity to take flight. A startle activity index was calculated giving more weight to those flies that arrived at the light.

Studies of sexual behavior were divided into female response to pheromone and mating propensity. Female response was measured in an olfactometer where air was blown across pheromone-producing males and into a large chamber containing virgin females. Respondents flew upwind to the pheromone source where they were captured in a cone trap and counted. Mating propensity was assessed in plexiglass cages where the number of mating pairs was recorded during each 10-min period for 60 min. A mating index was calculated by giving more weight to flies that mated faster and less to those that paired more slowly. Ratio tests using different strains were conducted in the same manner except that different strains or treatments were marked for identification.

The performance of fruit flies was also evaluated in field tests during the training course. Olfactometery and mating tests were conducted in large walk-in cages constructed of plastic screening. To measure female response to pheromone, small cages of virgin males were placed in a circular pattern on the floor of these field cages. Females were then released and collected every 15 min from the surface of the male cage to which they were attracted. Different strains, or treated and untreated

insects, were tested at the same time by differentially marking each group. Periodicity of pheromone production and of responsiveness by females was also determined. The field mating test was similar to that conducted in the laboratory. Virgin male and female flies (1:1) were simultaneously released into the field cage. At 15-min intervals during the 120-min test, observers inside captured mating pairs. The mating index and an isolation index were calculated. The latter was a measure of assortative mating when different strains were used and was calculated as the ratio of interstrain to intrastrain mating. Release-recapture tests measured the ability of the flies to disperse in the field. Flies were released from a central location and were collected in trimedlure-baited traps placed at varying distances from the release site. Estimates of the density of natural fly populations were determined using the resulting data. The uses of these tests during the training session in Spain and other countries were described by Boller et al. (1981) and Chambers et al. (in press).

A conference entitled "Advances and Challenges in Insect Rearing," chaired by R.F. Moore, was held in 1980. It brought together expertise in all areas of insect production, including genetic aspects of colonization, artificial diets, containment of insects, engineering, automation, control of pathogens and health hazards, management, and quality control. All papers impinged on quality insect production to a greater or lesser degree but

several dealt specifically with quality control. Chambers and Ashley (in press) advanced the use of process control charting to detect abnormal changes in production processes. A second paper, by Webb (in press), stressed that operational quality control can occur only when pertinent information on essential traits is returned to the production program in a typical feedback system. In addition, relevant production data (temperature, RH, lighting, procedures, diet) must be recorded to complete the feedback loop. Papers presented at this conference will be published as a USDA Technical Bulletin entitled "Advances and Challenges in Insect Rearing" edited by E.G. King and N.C. Leppla.

The quality control effort culminated last year with the formation of a Global Working Group on Quality Control under the auspices of IOBC. The workshop, held in Gainesville, Florida, was organized by T.R. Ashley, E.F. Boller, C.O. Calkins, D.L. Chambers, D.A. Dame, and N.C. Leppla. Discussions centered on pests of man and animals, fruit flies, lepidopterans, data management, and model systems. This workshop brought together scientists interested specifically in quality control, provided a comprehensive outline or frame of reference for unified discussions and coordination of future activities, and enhanced the stature of the quality control effort. A report on attempts to control the Mediterranean fruit fly in California illustrated the need for simple, standardized

tests and for organization of quality control efforts, especially in emergency situations. Major recommendations for future activities in quality control focused on genetic sexing and marking techniques; improved laboratory strains; data analysis, storage, and retrieval systems; distinguishing between genetic and non-genetic causes of quality reduction; verification of laboratory measurements of performance with field testing; correlation of easily measured variables with behavioral traits that are more difficult to quantify; development of reliable standards; and closing the feedback loop to ensure operational quality control. The conference concluded with inspection of the screwworm and Mediterranean fruit fly mass-production facilities in Mexico.

Factors Influencing the Quality of Colonized Insects

Quality of laboratory-reared insects may be influenced by the collection of founders, subsequent genetic changes, and environmental factors in the production facility. Collection strategies are based on the geographic, seasonal, and temporal distributions of wild insects. Their populations are not homogeneously distributed and areas of differential variability occur in relation to compatability with environmental conditions (Remington 1968). For example, in a constant environment, the optimal form of an enzyme will be the best functioning homodimer, while in an

environment where the probability of divergent conditions is greater, heterozygous individuals may be at an advantage (Johnson 1974). Thus, an enzyme in Drosophila melanogaster showed clinal variation with latitude. Alcohol dehydrogenase (ADH), which catalyzes the reduction of acetaldehyde to ethanol, is monomorphic in Florida where environmental conditions are relatively constant, but is more variable at the northern limits of the species (Vigue and Johnson 1973). Johnson (1976) also found clinal variation in polymorphic genes of Colias spp, a group of butterflies in the Pieridae family. He attributed these differences to different temperature optima for the isozymes.

As a result of these differences in distribution, founders should be derived from the target population in the environment into which future generations will be released and where they must survive. For example, wild corn earworm moths, Heliothis zea (Boddie), from the area of release in St. Croix, VI, were incorporated into existing laboratory cultures (Young et al. 1975). The new laboratory strain was more competitive than the old one from Georgia in finding and mating with wild females in St. Croix. In addition, males of the new strain remained more competitive even after six generations in the laboratory. In another example, a parasite of the walnut aphid, Chromaphis juglandicola (Kaltenbach), was collected in southern France, shipped to California, mass produced, and released throughout the

state. Those that survived did so only in areas near the coast where the climate was similar to that of southern France. For the hotter, drier areas parasites were obtained from a climatologically similar area of Iran. Subsequent production and releases in California were successful in controlling the aphid (Van den Bosch and Messenger 1973).

Large numbers of wild insects should be used to establish a laboratory colony to minimize the potential for founder effect that reduces genetic variability and results in genetic divergence from the source population. However, large collections do not always ensure colonization success, especially when the insects are taken from the more homogeneous marginal areas of their range, near the environmental limits of the species. Those selected from a more central location will tend to be outcrossed, with more heterogeneity (Remington 1968). Mackauer (1976) stated that a founder colony of about 500 individuals from a population of high average size plus long-term persistence, collected over a "conveniently" large area, should include a level of genetic diversity that adequately characterizes the parental population. In the tobacco budworm, Heliothis virescens (Fabricius), representative allele frequencies have been described using only 30-40 individuals, and therefore a colonizing population of 50-100 pairs should be adequate (M.D. Huettel pers. comm.). For establishing colonies that will ultimately interact with conspecifics in the field, MacDonald (1976) suggested that as many insects as possible

should be collected from the eventual target area, and collections should be made over several generations and during all discernable periods of daily activity.

Once founders have been introduced, other factors act to change the colony. Inbreeding results in a greater expression of harmful traits because of the increasing number of homozygotes for deleterious recessive alleles. Random genetic drift is a chance difference between gene frequencies of a parental generation and the ones represented by their progeny. The potential for drift depends on the size of the population, the selective value of the alleles (fitness), mutation pressure, and gene flow. Small, isolated populations like those in the laboratory are especially susceptible to drift and this may lead to fixation of one allele while its complement is completely lost from the population, regardless of its adaptive value (Herskowitz 1979). Under laboratory conditions, drift can result from a small founder population and periodic fluctuations in colony size (Mackauer 1976).

Directed selection for individuals best suited for the new conditions generally occurs in initial generations. During this period, the insects are forced through "bottlenecks" that alter and reduce the level of genetic variability (Boller 1972; 1976). The first bottleneck occurs in the larval stage in which mortality eliminates those individuals that cannot survive under the artificial regimen of diet, temperature, photoperiod, relative

humidity, density, and containment. The second occurs as similar artificial conditions reduce the reproductive potential of founders, as selection acts on adult behaviors that are directly related to insect quality.

Yields of colonizers are low at first when selection is most intense, but after about five to seven generations they improve as the number of laboratory-adapted individuals increases. For example, Raulston (1975a) found that newly colonized tobacco budworms mated less frequently and had a lower percentage of mating than insects in colonization for many generations. This difference in mating behavior, however, disappeared after six or seven generations in the laboratory. Similarly, Leppla et al. (1980) reported that an increasing number of matings and mating frequency of wild cabbage looper moths, Trichoplusia ni (Hubner), were indicative of progressive adaptation to laboratory conditions. They concluded that supernumerary matings may have contributed significantly to increased fecundity and, therefore, to success under laboratory conditions.

To reduce the effect of selection on initial generations, Boller (1972) suggested using rearing methods that give relatively high yields in the first generation and not selecting too early for a standardized strain. Further, various natural stimuli, including extracts of natural foods added to artificial diets, kairomones, and natural shape and color of rendezvous sites, etc., should be used to maintain specific behavioral traits. Finally, insects in culture

should be made to perform searching, orienting, flying, and mating behaviors in a relatively natural environment.

Laboratory rearing may result in other types of physiological deterioration that are often expressed as delayed maturation, increased mortality, and reduced fecundity (Mackauer 1972). This type of decline in quality has been described for the predaceous green lacewing, Chrysopa carnea Stephens (Jones et al. 1978). Developmental time of immatures increased, and egg viability, food consumption, searching ability, fecundity, and longevity of adults decreased with time in culture. No recovery phase occured in this species, and it was therefore recommended that insects intended for release should not be reared for more than six generations in the laboratory.

Genotypes or gene expression of laboratory-adapted individuals may differ from the original populations. For example, variation in diet can influence the allozyme patterns in insects (Sluss et al. 1978). Two groups of tobacco budworms reared on different artificial diets were found to have differences in allele frequencies at three loci, including two that code for hexokinases. These results were attributed to differences in sucrose content of the diets (hexokinase catalyzes the breakdown products of sucrose, glucose and fructose, in glycolysis). Gutherie and Carter (1972) found that larvae of the European corn borer, Ostrinia nubilalis (Hubner), reared continuously on artificial diet, lost the ability to survive on their

natural host. However, survival equaled that of wild borers after they were backcrossed to native insects. Whitten (1980) reported differences in allozymes at the alpha glycerophosphate dehydrogenase locus in the screwworm fly. The implication of this change is described below.

Environmental conditions in the laboratory may cause alterations in phenotype without associated changes in genotype. For example, density of insects in larval rearing containers reduces pupal weights in many species (Henneberry and Kishaba 1966; Sullivan and Sokal 1963; Barbosa et al. 1972). Peters and Barbosa (1977) have summarized the effects of density on fecundity, cannibalism, developmental rate, behavior, and disease susceptibility. Possible causes for density effect include mechanical stimulation by crowded individuals, amount and quality of food, and presence of insect-produced chemicals such as toxic factors and growth retardants.

The diet on which insects feed can alter growth, metamorphosis, reproduction, behavior, and defense (Hagen 1974 and references therein). Nutrient balance appears to be an important dietary factor. House (1965) found that larvae of the sphingid, Celerio euphorbiae (Linnaeus), feeding on an imbalanced diet ate less, were less efficient in food conversion, and gained less weight than larvae feeding on a diet of normal balance. Webster and Stoffolano (1978) reported that female apple maggot flies, Rhagoletis pomonella (Walsh) fed a protein/sucrose diet showed greater

development of ovaries, follicles, and accessary glands compared to a similar group given the same diet but without protein. Adult diets have been shown to influence the length of life for the codling moth, Laspeyresia pomonella (Linnaeus) (Howell 1981). Materials added to artificial diets also affect insect quality. Singh and House (1970) tested 21 agents commonly added to diets to reduce microbial contamination. Using larvae of a sarcophagid fly, Agria affinis auct. nec Fallen, they found that the length of larval development is inversely proportional to the dietary concentration of antimicrobial compounds. Successful pupation, adult emergence, and survivability were also adversely affected.

The effect of temperature on developmental rates of poikilotherms is well known (e.g. Butler and Henneberry 1976). Briese (1980) examined the effects of temperature on fertility, weight, survivability, fecundity, and sex ratio of the potato moth, Phthorimaea operculella (Zeller). Melanin formation in the integument of saltmarsh caterpillars, Estigmene acrea (Drury) was found to increase at higher rearing temperatures (Fye 1979). High temperatures were shown to cause abnormal wing development in the cabbage looper (Grau and Terriere 1967). However, this effect was moderated when the larval diet was supplemented with a source of polyunsaturated fatty acids (Dadd 1973).

Photoperiod and cyclical fluctuations in temperature can alter developmental and reproductive characteristics. For example, in continuous light, adults of the tobacco budworm and black cutworm, Agrotis ipsilon (Hufnagel) showed increased irritability, inability to separate after copulation, a shortened life span, and a decrease in fecundity. Two days after establishment of a 14 hr light:10 hr dark photoperiod, normal activity returned (Fisher unpublished). Messenger (1964) reported that a laboratory strain of the spotted alfalfa aphid, Therioaphis maculata (Buckton), held under fluctuating temperature developed faster and had improved fecundity and adult longevity compared to a similar group reared under constant conditions. On the other hand, larvae of a laboratory strain of the corn earworm reared under constant temperature developed faster but had lighter pupae and greater fecundity than a group reared under fluctuating conditions (Fisher unpublished).

Rearing procedures and post-production treatments can affect adult behavior and cause reductions in insect quality. For example, the process of agitation used to separate puparia from their diet caused a droopy-wing syndrome in colonies of Mediterranean fruit flies, Oriental fruit flies, Dacus dorsalis Hendel, and melon flies, D. cucurbitae Coquillet (Ozaki and Kobayashi 1981). The syndrome resulted in reduced eclosion rates and increased numbers of non-fliers as intensity and duration of agitation

increased. Five-day-old puparia were found to be the most sensitive to agitation (Ozaki and Kobayashi 1982). The detrimental effects are well known for treatments, such as sterilizing doses of irradiation or chemosterilants, on the mating competitiveness of laboratory insects (Noblet et al. 1969; Snow et al. 1972; Brower 1978; and Villavaso 1981), sperm transfer (Holt and North 1970; Snow et al. 1970), and flight (Shepard et al. 1968). However, to decrease rearing costs, dieldrin was added to the larval diet of the calliphorid, Lucilia cuprina (Wiedemann), to selectively kill females that were of no use in an autocidal release program. Males were protected by a Y-autosome translocation for dieldrin resistance. Laboratory assays of flight activity (actograph), sexual competitiveness (ratio tests evaluated by number mating), and visual sensitivity (ERG) showed no differences between treated males and internal standards, but treated males did not disperse as well when released in the field (Smith et al. 1981).

Comparison of Colonized Insects With Wild Standards

Tests have shown a wide disparity between laboratory and wild insects. To illustrate, White and Mantey (1977) observed that male laboratory codling moths were more sedentary than wild ones in a field release. Similarly, the suspected inability of laboratory screwworm flies to disperse in the field was cited as the reason for reduced

effectiveness of a release program in the mid-1970's (Bush 1979). This problem was attributed to rearing at a high, constant temperature to increase production efficiency. Selection for a laboratory-adapted electromorph, GDH2, occurred in preference to GDH1, the form that occurs almost exclusively in wild flies. This enzyme is critical in the transfer of reducing equivalents to the glycerol phosphate shuttle during energy production in flight muscles. As a result, released flies were unable to disperse and mate. Sharp (1976) reported differences in time of flight, distance flown, and flight velocity between a wild strain of Caribbean fruit fly, Anastrepha suspensa (Loew), and one reared in the laboratory for more than 80 generations. However, flight ability of the wild strain declined to the level of the laboratory strain by the third generation. Lab-adapted cabbage looper larvae dispersed less than wild larvae in field cages (Leppla and Guy 1980).

The diel periodicities of laboratory insects may differ from their native counterparts. In a field release of tobacco budworm adults, Raulston et al. (1976) recovered twice as many wild as laboratory males in traps baited with virgin females because laboratory males became active 2 hr later. Turner et al. (1977) found that wild cabbage looper moths respired nearly twice as much carbon dioxide compared to laboratory moths. Wild insects had a bimodal pattern of nocturnal activity, while laboratory moths were unimodal. It was suggested that this reduced activity by laboratory

insects during scotophase may mean reduced dispersal capacity, host-seeking capabilities, and mating competitiveness with the field population. On the other hand, a lower metabolic rate in the laboratory strain may have resulted in increased fecundity, fertility, and survival.

Visual sensitivity may decline in colonized insects. Agee and Chambers (1980), using ERG, found wild Mediterranean fruit flies that developed on fruit as larvae required 0.1 microwatt/cm^2 of light energy to stimulate a response, whereas a group of laboratory flies reared for four generations on unnatural bagasse diet required a stimulus of about 0.65 microwatt/cm^2. Vision declined further if mold occurred on the diet or if agar replaced corncob meal. Histological examinations revealed differences in the ultrastructure and cellular organization of the ommatidial units of the compound eyes, with the rhabdomers of the wild flies well organized and full bodied. Conversely, those of the laboratory flies were smaller in diameter with larger inter-rhabdomeric spaces (Agee and Davis unpublished). Goodenough et al. (1977) observed that the vision of mass-reared screwworms was three times less sensitive than that of wild flies.

Pheromone production and response to it also may be altered during colonization. Fletcher et al. (1968) found that females of an 11-year old colony of screwworms responded much more aggressively to pheromone extracts than

the recently colonized strain. Selection pressure for 11 years probably favored a colony of flies that readily mated in dark, crowded conditons. Pheromone responses of male gypsy moths, Lymantria dispar (Linnaeus), reared for one or more generations in the laboratory on artificial diet were compared with moths reared from field-collected pupae (Richerson and Cameron 1974). In laboratory bioassays, wild males were more responsive to disparlure than were laboratory males. In a field cage, wild males were more successful in orienting to pheromone produced by wild females than were laboratory males. Also, a greater number of wild males made initial contact with the female, regardless of whether she was laboratory-reared or wild. Finally, in choice tests, both laboratory and wild males overwhelmingly selected wild females for mates. In another study, however, Waldvogel et al. (1982) found that male laboratory gypsy moths responded to a synthetic pheromone source in the same way as wild moths. Lab females, however, showed no periodicity of emission and a large number failed to release any detectable quantities of pheromone. Conversely, wild females showed a diel periodicity of pheromone emission (Richerson and Cameron 1974).

Reduced pheromone production was observed by Miller and Roelofs (1980) using the red-banded leafroller, Argrotaenia velutinana. They found that females from a strain reared for 30 years in a greenhouse produced less pheromone than did wild females, although body weights were the same.

Also, the percentage of (E)-11-tetradcenyl acetate in the pheromone blend was lower in the laboratory strain. Minks (1971) also found alteration in pheromone production in an inbred stock of the summerfruit tortrix moth, Adoxophyes orana (F.v.R.). Two strains were colonized simultaneously and reared on similar artificial diets. One strain was infused annually with 100-200 wild insects, produced 500-1500 moths per month, and received ascorbic acid in the larval diet. The second strain was inbred, was reared at greater densities to produce 500-1000 moths per day, and did not have vitamin C in the diet. In field assays using traps baited with virgin females from each strain, more wild males were captured by females from the first strain than by those of the inbred strain. Results of laboratory bioassays indicated that the extractable pheromone content in the in-bred line was much lower than that of the other females. Legget and Moore (1982) found that the number of female boll weevils, Anthonomis grandis grandis Boheman, responding to grandlure traps was a function of the larval or adult diet. Either high protein or low sugar or the combination of high protein-low sugar was associated with increased response to the traps.

Laboratory rearing may affect mating behavior and reproductive physiology of colonized insects. Fye and LaBrecque (1966) demonstrated that wild and laboratory female house flies, Musca domestica Linnaeus, mated more frequently with males from their own strain. LaChance et

al. (1975) found that native male pink bollworms, Pectinophora gossypiella (Saunders), transferred normal amounts of eupyrene (nucleated) sperm more often than a strain reared for nearly eight years on artificial diet. The duplexes of wild males contained more eupyrene sperm bundles which apparently left the testis and descended to the duplex region more rapidly than in laboratory males. However, laboratory males were still able to inseminate females with adequate amounts of sperm. Codling moth males reared in the laboratory for four years and released in the field were observed to mate less frequently with wild females than did wild males (White and Mantey 1977). Males of another lepidopteran, Adoxophyes orana (F.R.) reared in the laboratory for six years and irradiated as adults were only 0.58 as competitive as wild males in tests conducted in cages placed over apple trees (Denlinger et al. 1973). Because no difference was demonstrated between laboratory and wild males reared for one generation on artificial diet, the authors concluded that the laboratory males were not genetically inferior, but that the rearing procedure, probably the artificial diet, caused the decline in competitiveness. Henneberry and Clayton (1981) reported that irradiated and unirradiated laboratory-reared male pink bollworm moths failed to mate as frequently with native females as with laboratory females. On the other hand, laboratory females, treated or untreated, mated with equal frequency to either laboratory or wild males.

Oviposition behavior of insects may be modified in a laboratory environment. Greany and Szentesi (1979) compared ovipositional responses in wild and laboratory Caribbean fruit flies and found that wild flies selected black domes almost exclusively, but laboratory flies showed no preference between black and white ones. Wild flies showed an almost absolute preference for dome-shaped substrates, whereas laboratory flies deposited 12% of their eggs onto flat discs. This unnatural behavior may have been caused by selection for females that oviposit on flat surfaces provided for them in the rearing program. Because many tephritid flies use fruit as a rendezvous site for mating, a change in fruit-finding capability by laboratory flies could adversely affect their mating competitiveness.

Longevity of males from a colonized strain of tsetse fly, Glossina moristans orientalis Vanderplank, was significantly less than that of a wild strain (Dame et al. 1970). The differential mortality apparently resulted from reduced feeding activity of laboratory flies in holding cages that were larger than those in which they had been reared. Wild flies fed normally in the large cages.

Identifying and Interpreting Changes in Insect Quality

To monitor the changes in colonized insects, the use of industrial quality control procedures has been proposed. The most important of these is the process control chart,

developed by Shewart (1931), which employs statistical methods to evaluate changes over time. Such charts are based on separating variation in sample populations into a random component, or unidentified variation inherent in anything being measured, and a component that results from specific assignable causes. To construct a quality control chart, enough samples are taken to determine random variability (standard deviation = s.d.) and distribution. With this information, the significance of a change in quality can be determined. Even if the distribution is not normal, constants used to determine chart characteristics are so stable that those for normal curves may be used unless the distribution is extremely skewed (Burr 1967). The chart consists of a center line, the overall mean of the samples, and the control limits, usually $\pm$ 3 s.d. units, which are marked above and below the center line. The ordinate is the value of the trait being measured and the abcissa is successive sample numbers.

If only chance variation is present, no definite patterns appear on the chart over time and values are balanced above and below the center line. But, if an assignable cause is altering the degree of variability, sample values will fall outside the distribution limits and it can be assumed, with a high degree of confidence, that they came from populations treated differently. Such values are said to be out of control.

Several types of charts are commonly used in industry.

Average ($\bar{X}$) and range (R) charts show variation in sample averages and ranges. These charts, often displayed together, utilize variable data and require relatively small sample sizes to give good results. Measurement of variable data, however, may be time consuming. P-charts are used for discrete or attribute data and show the percentage of sample that is defective or that must be rejected. These charts use data that are easily measured by a yes or no response but they do require large numbers of observations for accuracy. Operating characteristic, or OC charts define the sensitivity of a control chart for detecting changes in processes or product quality.

Because standard deviation varies inversely with $\sqrt{n}$, larger sample sizes increase the sensitivity of the chart by moving limits closer to the control line. But large samples are often expensive and sampling strategies should be balanced between economy and the cost of an undetected shift in processes. Generally, if it is more costly to sample and test than it is for a change in product quality to go undetected, it is better to take small samples more frequently. Samples must be taken from production output that has been treated similarly. Thus, if assignable causes are present, the differences will be apparent between samples rather than between observations within a sample (Duncan 1959).

Interpretation of control charts begins with identification of out-of-control points and upward or

downward trends. If this occurs on an $\bar{X}$-chart, it means a general change in processes has affected all samples. Similar points on an R-chart show a change in process uniformity. A run of non-randomness is indicated by four out of five successive points beyond 1 s.d., two out of three points beyond 2 s.d., or eight successive points on one side of the center line (Bicking and Gryna 1974). Process control charts provide information as to when, and to what degree, modifications of processes should be made, and they assist in identifying factors in production that affect product quality (Charbonneau and Webster 1978).

Chambers and Ashley (in press) emphasized that quality control is an active approach to maintaining and improving insect quality through control of processes. A process is the interaction of people, materials, equipment, and facilities required to make a product. Thus, diet preparation, egg surface-sterilization, larval development, and pupal harvest procedures are examples of processes in a rearing program. Processes can also include colonization and infusion strategies designed to ensure adequate genetic variability. Total quality control is based on a systematic approach to solving insect production problems. It works best when thoroughly integrated with the production program and its effectiveness is dependent on communication between producers and users.

The basic steps in establishing a quality control program have been outlined by Boller and Chambers (1977) and

Leppla et al. (1977). First, objectives of the rearing program are defined by characterizing the target population, prioritizing critical behaviors, and determining production capacity and schedules. Next, standards are established against which insect quality is measured. They should be precise and descriptive of both the average and range of acceptable performance levels for important performance traits. For more complete analysis of overall quality, standards should be established for various components of physiology, morphology, genetics, and behavior. Then, methods to quantify these traits are developed. They should be simple, economical, reproducible, and able to be performed by anyone. Finally, the quality control program is implemented with regular monitoring and the use of process control charts to provide the feedback necessary to ensure production of quality insects.

Characteristics of Lepidopteran Rearing

Mass production programs for lepidopteran species can be characterized by the composition, preparation, and methods of dispensing artificial diets; containerization; procedures for handling insects; facility design; and quality control. Traditionally, larval diets have consisted of a mixture of primary ingredients such as wheat germ, casein, bean meals, Brewer's yeast, and secondary materials such as carbohydrate and unsaturated fatty acid sources,

vitamins, minerals, and antimicrobial agents in an agar suspension (Singh 1977). The expense of many of these ingredients, however, limited large-scale rearing, but the elimination, substitution, or reduction of the expensive ones has allowed more economical production. For example, casein was replaced with wheat germ (Raulston and Shaver 1970) or soy flour (Stewart in press), corncob grits reduced the agar requirment (Brewer and King 1979), and the concentration of vitamins was reduced without detrimental effects on insects (Raulston 1975b; Brewer and Tidwell 1975). The use of tetracycline and formalin in two artificial diets for the beet armyworm, Spodoptera exigua (Hubner), and tobacco budworm was eliminated and the concentration of primary ingredients was significantly reduced without altering insect quality (Fisher unpublished). Toba et al. (1970) proposed a rating system to evaluate the suitability of diets modified to reduce costs. Criteria such as time to pupation, pupal yield, percentage of emergence, fecundity, and fertility were used to calculate an average suitability value expressed as a percentage of the results achieved with the control diet.

Artificial diets are prepared in mixers or steam kettles with capacities from 4 to 775 liters. Ingredients may be sterilized prior to mixing to kill microbes, render certain toxic components inactive, reduce moisture content to improve storage properties, and concentrate nutrients (Vanderzant 1974). Other systems employ a

flash-sterilization technique in which diet is moved through small-diameter tubes, quickly heated to 145-160° C, and sterilized within two to three minutes (Griffin et al. 1974). The diet is then pumped directly to a station where it is aseptically dispensed into rearing containers. This system is in use at the USDA facilities, Stoneville, MS, for the production of Heliothis spp. (Gantt et al. 1977).

Requirements for larval containers are determined by the species being reared and its habits (Burton and Perkins in press). Cannibalistic species must be reared individually or at low densities. Glass shell vials, disposable 30-ml plastic cups, or 0.2 to 0.5-liter cardboard cups are often used and eggs or neonate larvae are usually transferred by hand to each container. Burton and Cox (1966) used a food packaging machine to dispense 30-ml cups, fill each with diet, add larvae, and cap them at a rate of 2,000 to 4,500 per hour. But, because of the cost of cups, caps, and labor, these containers were unsuited for mass-production. Thus, larger, reusable containers were employed. For example, a 6-liter, round, plastic container was developed to rear 8,000 to 10,000 codling moth pupae per day. To reduce dessication, the diet was lightly brushed with melted paraffin that was perforated with small holes to allow neonates to reach the diet. Pupation occurred in corrugated cardboard strips taped to the perimeter of the container (Fisher in press, a). Rye et al. (1981) described a similar, but rectangular container for weekly production

of about 6,000 cabbage looper pupae. To further improve production efficiency of cannibalistic tobacco budworm larvae, Raulston and Lingren (1972) used polystyrene light-diffusing louvers imbedded in diet in fiberglass trays. Each rearing unit consisted of individual 1.2-cm rearing cells and could accomodate over 900 larvae. An aluminum template, with rows of small depressions corresponding to the location of the cells, was used to add eggs. Loose eggs poured onto the template were gently shaken until three to four filled each depression. After excess eggs were removed, the cell unit was inverted over the template, both were reinverted together, and the eggs were gently tapped into the cells. This process has been automated using a conveyor belt to move diet trays under several small tubes aligned over the rows of cells. A hopper located above, metered eggs into each tube at a rate of two to three eggs per cell. Polypropylene cloth was then placed on top of the cell unit to allow air flow and prevent larval escapes (Harrell and Gantt in press). This type of tray rearing has been used to produce over 35,000 tobacco budworm pupae (Raulston and Lingren 1972) and 60,000 Heliothis spp. pupae (Hartley et al. 1982) per day. In the latter program, the material covering the cell unit was changed to a porous, autoclavable polypropylene sheet. However, mold contamination was a problem with tray rearing because steam autoclaving melted the cell units and disinfection solutions were inadequate to achieve

sterilization. Therefore, a cell unit was made of heat-stable casting resin that was easily cleaned and sanitized (Fisher in press, b).

An automated containerization system, developed for the corn earworm was described by Sparks and Harrell (1976). An in-line, form-fill-seal machine pressure formed high-impact polystyrene film into a continuous sheet of rearing cells, dispensed artificial diet (Harrell et al. 1973) and insect eggs (Harrell et al. 1974) into each cell, sealed the top with plastic film, and cut the sheet into 4 by 8-cell sections for stacking in holding rooms. The capacity during continuous operation was projected to be 160,000 cells per 8-hour day. For the production of pink bollworm, a non-cannibalistic species, in yields of two million per day, diet was dispensed into trays and stored for three days to dissipate volatiles and reduce the moisture content (Stewart in press). The diet was then shredded in commercial equipment to increase its surface area for feeding and separating larvae and placed into larval rearing containers.

Various techniques were used to harvest insects from larval rearing containers. For example, late instar pink bollworm larvae crawled out of the containers and dropped below to honeycomb cells where they pupated. In other species, pupae are harvested directly from the containers. In small and medium-sized containers, workers usually collect pupae with forceps. However, Harrell et al. (1968) designed a machine to mechanically harvest fall armyworm

pupae from 30-ml cups. The cups were placed on a conveyor belt and crushed to remove lids and dislodge pupae which were separated from the debris and collected. About 4,000 cups were harvested in an hour, an improvement of 600% over manual collection. This machine was slightly modified to harvest corn earworm pupae as well (Harrell et al. 1969). Polystyrene and resin cell units were simply inverted and pupae fell into a tray. Pupae produced by the form-fill-seal machine were also mechanically harvested. The plastic film covering the cells was stripped away and the sections were inverted over a wire conveyor belt that allowed pupae to fall through to a collection tray and that deposited debris in a trash receptacle. The capacity was 20,000 to 25,000 cells per hour. Harvesting other lepidopteran pupae may require additional steps. For example, the cabbage looper pupates in silken cocoons on the top and sides of the container. After harvest, the pupae are placed into a dilute solution of sodium hypochlorite to remove the silk (Henneberry and Kishaba 1966).

Adult eclosion, mating, and oviposition usually occur in a single cage, its characteristics being determined by availability of materials, ease of handling, and reusability. Cardboard ice-cream cartons (3.8 liters) are commonly used for adults (Shorey and Hale 1965). Oviposition materials such as nylon organdy, cheesecloth, or paper toweling are placed over the top of the cartons with narrower pieces drapped down the sides. Other cages are

made of wooden, stainless steel, or aluminum frames covered with screen or clear plastic (Ignoffo 1963). Automated cages have been developed to minimize disturbance to moths and reduce time required to harvest egg sheets (Knott et al. 1966; Carlyle et al. 1975). For the mass-production of 100,000 Heliothis spp. eggs per week, windowscreen mounted in an aluminum frame was inserted through a slot in the cage and used as an oviposition substrate. Its replacement does not disrupt adults or require that they be anaesthetized (McWilliams et al. 1981). In pink bollworm rearing, emergence occurs in a container separate from the one used for mating and oviposition. Newly-eclosed adults are attracted to a black light and are picked up in an airstream that transports them to collection centers in a walk-in refrigerator where cool temperatures reduce their activity and permit easy transfer to new cages for mating and oviposition.

Air-borne contaminants such as wing scales and hairs represent health hazards to insectary employees and help disseminate disease organisms (Stewart in press; Wirtz in press). Thus, various scale-collecting devices have been constructed. Raulston and Lingren (1972) placed adult cages on top of a hollow shelf attached by plastic piping to a blower which gently pulled air through the cages and into a filtration unit. Pink bollworm cages were attached to a series of pipes that pulled air from the cages to cyclone dust collectors located outside the building. Leppla et al.

(1982) described cabinets that enclosed adult cages and removed scales using an industrial dust collector. Absolute filters in the air handling system of the gypsy moth facility removed scales from the air while individual work stations were equipped with exhaust hoods and a vacuum to further reduce this hazard (O'Dell et al. in press).

The production facility is the primary component of a successful rearing program (Leppla and Ashley 1978). Its design is intended to maintain environmental conditions, confine insects, control pathogens and dietary contaminants, isolate specialized areas, allow efficient movement of materials and products, and provide a proper working environment (Fisher and Leppla in preparation). Design criteria are especially important for lepidopteran facilities because most species are susceptible to a variety of insect pathogens. In fact, Stewart (in press) stated ". . .in every instance, failure to meet production quotas [of pink bollworm] was directly related to the dominating, detrimental effects of microorganisms". Also, Sparks and Harrell (1976) indicated that the inability of a facility design to maintain disease-free insects is a major cause for failure when the production capacity is increased. The most effective means to eliminate disease outbreaks is to isolate critical areas. Thus, preparing diet in an area completely separated from other rearing activities was the only practical way to eliminate virus and bacterial diseases from colonies of tobacco budworm and beet armyworm (Fisher in

press, a). Raulston and Lingren (1972) designed a facility for tobacco budworm that completely isolated the brood colony from the mass-production area, provided showers for incoming employees, and incorporated pass-throughs and a double-door autoclave for the movement of materials and products without a backflow of contaminants. In addition, all rooms except the one for pupal harvest, the dirtiest, were maintained under positive pressure to exclude contaminants.

To complement facility design, procedures have been developed to reduce disease outbreaks. These include addition of antibiotics to larval and adult diets, surface-sterilization of eggs and pupae with dilute solutions of sodium hypochlorite or formaldehyde, regular sanitation of the facility, regulations restricting unnecessary movement of personnel, and maintenance of environmental conditions to reduce stress on the insects (Sikorowski 1975; in press).

The need for quality control in lepidopteran rearing programs is clear but usually only consists of monitoring pupal yields and weight, fecundity, and temperature and RH in insect holding areas. Other variables, although more meaningful in quality assessment, are measured in only a few programs. For example, the quality of mass-reared gypsy moths was evaluated in laboratory and field tests (O'Dell et al. in press). During production, routine measurements were made of survival, eclosion, deformity, and fertility. A

flight tunnel was used to determine male response to pheromone by measuring the length of wing-fanning periods and the time of flight in a pheromone plume. Data from actographs, used to identify activity periods and assess propensity to fly, were correlated with the pheromone-response rhythm of released moths. Observations of mating behavior were made to determine the optimal pupal age and radiation dosage for obtaining sterile males while maintaining sexual competitiveness. In the field, measurements were made of eclosion success, the periodicity of eclosion, and longevity. Also monitored was the number of laboratory males responding and dispersing to pheromone-baited traps. The production of Heliothis spp. hybrids was monitored at the production facility using fertility of untreated eggs, surface-sterilized eggs, and the weights of pupae before and after harvest (Brewer in press). Other variables measured included pupal diameter, abdominal rotation, mortality, percent adult emergence and wing deformity, and longevity. At St. Croix, VI, postdistribution tests of the hybrids included periodicity of activities such as feeding and flight, dispersal, mating competitiveness using mating tables (Snow et al. 1976), and oviposition (Proshold 1982). For the pink bollworm, tests of mating ability and longevity identified detrimental effects of handling and shipping and evaluated the potential usefulness of moths in the field (Stewart in press).

Virtually all of the quality control tests for

lepidopterans were conducted on a short-term research basis and were not used to continuously monitor production or product quality. In fact, procedures for lepidopteran mass rearing are so time consuming and labor intensive that they generally leave little time for quality assessment. Thus, a system is needed that will continuously provide basic data on insect quality in an economical and reliable manner. Accordingly, the objectives of this study were to: 1) identify developmental, morphological, and behavioral traits that could be used to monitor production processes and to indicate the relative quality of a lepidopteran species reared in the laboratory; 2) design and construct equipment to measure these traits; 3) extract information on behavior patterns as well as the results of behavior; 4) determine the efficacy of the testing system; 5) modify rearing conditions to determine if resultant changes in quality can be detected; and 6) incorporate these and other data into a model quality control system for mass-reared Lepidoptera.

METHODS AND MATERIALS

Source of Insects and Rearing Conditions

The fall armyworm, Spodoptera frugiperda (J.E. Smith), was used for this study. It is a significant economic pest in the southeastern United States beginning in April that affects much of the rest of the eastern part of the country after July (Sparks 1979). Average losses exceed $300 million annually and $500 million during years of severe outbreaks (Mitchell 1979). Fall armyworm larvae are polyphagous, feeding on a diversity of crops such as grasses, soybeans, corn, millet, alfalfa, rice, cotton, and peanuts (Young 1979). This species lacks a diapause mechanism and its only overwintering sites in the United States are in the milder climates of south Texas and Florida (Sparks 1979). Thus, it is an ideal candidate for a regional approach to early season control using sterile insect technique (Knipling 1979, 1980).

Fall armyworm eggs are laid in masses on the underside of leaves. However, in high population densities they have been deposited indiscriminately on plants, buildings, and other objects (Thomson and All 1982). Masses are covered with scales and setae from the body of the female. After

hatch, neonate larvae eat their eggshells and, in response to positive phototaxis and negative geotaxis, climb to the uppermost portions of the plant where they secrete a line of silk to aid wind dispersal or begin feeding on tender terminal growth (Luginbill 1928; Morrill and Greene 1973). Early-instars skeletonize leaves of host plants while later ones eat the entire leaf, often stripping the entire plant. Feeding occurs at night and larvae hide at the base of plants under debris during daylight hours. Prepupae are positively geotactic and usually pupate in the soil, although pupae have been found in stalks, ears, tassels, and whorls of corn (Burkhardt 1952). Adult eclosion begins shortly after sunset and continues until midnight and feeding on nectar is apparently the only activity performed during the first night (Sparks 1979). On subsequent nights, activity of moths begins about dusk. After an initial feeding period, females begin calling from a location near the top of the plant canopy. In response to the pheromone, males orient their antennae toward the source, vibrate their wings, and fly in the direction of the female (Sekul and Cox 1965). In other cases, males orient to the attractant on the wing as they fly obliquely upwind. Often several males respond to the same female, rejected males returning to flight, sometimes in large groups (Sparks 1979). Mating behavior of fall armyworm moths has not been reported but, apparently, males and females mate only once each night and most mating occurs by midnight. Eggs are deposited during

the early evening. In summer, and at 27° C in the laboratory, the life cycle is completed in about 30 days.

Lab fall armyworms were produced at the Insect Attractants, Behavior, and Basic Biology Research Laboratory, U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS) in Gainesville, Florida, where they had been in production for six years without infusion of wild stock. Original founders consisted of about 300 insects from the Southern Grain Insects Research Laboratory, USDA, ARS, Tifton, GA, where they had been in culture for 15 years (Perkins 1979).

At Gainesville, larvae were reared in 30.5 x 30.5 x 14-cm high plastic containers with snap-on lids modified to improve air exchange (Rye et al. 1981). Each container received 1000 g of a pinto bean-based artificial diet (see appendix for ingredients). A polystyrene grid of 1.3 x 1.3-cm squares was pushed into the diet before it cooled. This grid separated developing larvae and facilitated the harvest of pupae. Paper toweling, covered with fall armyworm egg masses, was glued to the inside of each container lid. Four containers set up each work day were dated and labeled according to rack 1 or 2 and location on a shelf. Locations were designated A, B, C, or D with "A" on the left end of each shelf.

Larval rearing containers were maintained at 26° ± 2 C and 50 ± 5% RH with a 14 hr light:10 hr dark cycle. After 21 days, pupae were harvested and the approximate amount of

diet surface covered by mold and the number of insects was determined. The number of insects was divided into three categories: usable pupae, those that were normal looking and from which normal adults would probably eclose; abnormal pupae, identified by some morphological deformity such as larval exuvia attached to the venter or a constricted abdomen; and non-pupae, defined as larvae, or insects with a pupal-like anterior portion and a larval abdomen. Total number of insects was the sum of all insects in these categories at the time of pupal harvest. After yields were determined, usable pupae were held for quality control testing. Twenty-five pupae were randomly selected from 151 containers between 24 August and 5 November 1981, separated by sex, and held for limited testing (pupal weight only), and between 70 and 150 pupae of each sex were taken from each of two containers randomly selected on 36 harvest days between 20 April and 30 July 1982 and held for comprehensive testing.

Holding Conditions for Test Insects

In 1982, 30 to 90 pupae from each larval container were set up in 3.8-liter cylindrical cardboard holding cages with nylon organdy covers. When more than 70 pupae were set up, they were divided approximately equally into two replications. Males and females were placed in separate 107 x 58 x 46-cm-high plywood cabinets while pairs used in

mating studies were held in a third. Conditions within cabinets were 28 ± 2° C and 67 ± 12% RH. Humidity was maintained individually for each cabinet by centrifugal atomizers. Lighting, provided by one 30-watt cool white fluorescent tube per cabinet (480 - 590 lux), was on a reversed cycle of 14 hr light:10 hr dark with scotophase beginning at 10:30 am EST. This enabled testing and observations of insects during their most active period, between 2 and 4 hr after initiation of the dark cycle (Leppla et al. 1979). Twilight conditions were provided by a poultry timer (Grainger Model 2E023) that operated small 0.25-watt nightlights for 20 min at the end of photophase. Small fans, used for cooling electronic equipment, operated during the light cycle to remove heat produced by lights. All cabinets were located in a controlled environmental room.

Insects in holding cages were checked twice daily for adult eclosion and aging of adults began when 25% had emerged. Adults received 10% sucrose/water solution in cotton placed on top of the organdy covering the cages. When all testing was concluded, cages were removed from cabinets and counts were made of the number of dead pupae, the number of unsuccessfully eclosed adults (those unable to completely separate abdomen or wings from exuviae), and adult deformities (wing curl).

Characteristics of Immature Insects

All additional pupal data were taken on 5-day-old insects. In 1981, average weights were calculated using variable sample sizes while, in 1982, individual weights were determined for 20 male and female pupae from each container. Abdominal rotation was determined from another group of 20 pupae using a protractor-like device (Fig. 1). To support the pupae, a rubber trough was attached by mastic to an 11-cm diameter clear plastic disk. Lines, in increments of ten degrees radiated from the midpoint of the end of the trough. A pupa was placed in the support trough with its ventral side up and its abdominal segments projecting over the edge. Slight pressure on the thoracic region stimulated the abdomen to move in a circular motion and the maximal amount of movement to the left and the right in ten rotations were added to get the degree of abdominal rotation.

The inverse time of pupal descent in water, a measure of buoyancy, was determined for 20 male and female pupae from each container. A 250-ml graduated cylinder was filled with 24° C water to which one drop of surfactant (liquid dishwashing detergent) had been added. Using forceps, a pupa was placed in the water and slightly shaken to remove air bubbles adhering to it. The pupa was then released and allowed to descend to the bottom of the cylinder. Timing with a stopwatch started when the pupa passed the 230-ml

mark and ended when it crossed the 70-ml line, a distance of 15.3 cm. Pupae were caught at the bottom in a 3.0 x 5.0-cm cylindrical cage made of 3.0-mm-mesh hardware cloth. A wire attached to the cage was used to pull it out of the graduated cylinder.

In preparation for all adult tests, male and female moths were transferred from holding cages to vials 1/2 to 1 1/2 hr before the end of photophase. At the beginning of scotophase, vials were placed in the dark where they remained until testing. Temperature was 25 ± 1° C and RH was uncontrolled at about 50%. Lighting varied and is discussed under individual procedures.

Testing of Adult Insects

Wingbeat frequency

Wingbeat frequency (WBF) was determined using a stationary vacuum tethering device (Fig. 2). Moths were attached to the distal end of a 100-microliter pipet. Surgical tubing connected the proximal end of the pipet to a vacuum source via two needle valves. Valve A, closest to the vacuum source, remained open a precise amount to ensure constant pressure. Valve B was used to shut the vacuum off after each insect was tested. Vacuum pressure at the distal end of the pipet was about 339.9 g/cm^2. The vacuum tethering device was a non-destructive means to determine WBF. That is, unlike quick-setting glues, vacuum did little more than

more than remove a small patch (about 1.5 mm in diameter) of hairs and scales from the area of attachment, thereby enabling the insect to be used for other quality control tests. Illumination during this test came from a yellow 25-watt incandescent bulb mounted 30.5 cm above the tethered insect and was controlled by a rheostat to about 13 lux. A stroboscope (General Radio Strobotac Model 1538A) was used to determine WBF.

In preparation for testing, 5- to 7-day-old moths were placed in 20-ml glass vials, chilled in chopped ice for 2.5 min to slow their activity, emptied into a 5-cm-diameter watchglass, and oriented with the dorsal side up and the anterior end facing the observer. After the vacuum was turned on at valve B, the watchglass was lifted and the moth was slowly brought into contact with the pipet. Attachment occurred at the center of the metathorax so the longitudinal and transverse axes of the insect were perpendicular to the pipet. A plumbob was used to ensure that the pipet was perpendicular to the ground. Timing with a stopwatch began when the moth started beating its wings, usually immediately after tarsal contact was lost when the watchglass was removed. After 30 sec, the stroboscope was turned on and WBF was taken 1.0 min later. Then, valve B was closed, the moth dropped into an empty vial, and another moth was tethered. Replications consisted of five to ten moths.

Response to pheromone

This test was conducted in a flight tunnel made of clear acrylic similar to that described by Miller and Roelofs (1978) (Fig. 3). An 11-cm-diameter fan pushed air through a baffle system consisting of two partitions of 76-mesh nylon organdy material. These diffused the air and provided a uniform flow down the length of the tunnel at a rate of 24 ± 2 cm/sec. Females in wire cages were placed in a chamber between the last baffle and the flight tunnel proper, being separated from the latter by a 20-mesh screen to keep males from entering the chamber. A 12.0 x 12.0-cm plastic target was located on the screen to frame the pheromone plume produced by the females. At the opposite end of the tunnel, a 14.0-cm-high stand held the release cage in front of a 10.2-cm hole through which pheromone-laden air was exhausted outside the building via flexible hose. A clear plastic starting gate, attached to a control rod, was used to keep males from leaving the release cage before the observer was ready to begin the test. Males were removed from the tunnel with a suction device at the completion of each run. Lighting was provided by two 15-watt red (greater than 600 nm) fluorescent tubes located behind the tunnel. The back wall and ends of the tunnel were covered with light-diffusing material (Armstrong 420A) that provided backlighting to silhouette insects, enabling precise observation without altering their behavior. Light intensity was about 11 lux.

Four- to 5-day-old wild and laboratory males were placed individually into release cages made from 30.0 x 55.0-mm plastic dilution vials each with a 19.0-mm-diameter hole drilled in the bottom and covered with aluminum window screen to allow air to flow through them. Prior to testing, the fan was turned on in the tunnel and two females were placed in each of two wire cages that were mounted on 11-cm-high screen pedestals in the center of the chamber. Females began calling within ten minutes when a release cage was placed on the stage with its open end directed upwind and covered with the starting gate. The gate was pulled away from the cage when the observer was ready to begin the test.

Response to pheromone was first indicated by clasper extension inside the release cage. Timing by stopwatch began as soon as the male left the cage. Moths were observed for directed flight (anemotactic flight for more than 3 sec in the pheromone plume) and were scored as to whether or not they landed inside the plastic target. The amount of time it took a male to arrive at the source, whether by flying or walking, was recorded. Also, the time spent in flight was recorded using a second stopwatch. Tests were terminated when a male arrived at the target or at the end of three minutes. Eight to ten males were tested per larval rearing container. After each test day, all components of the tunnel system were cleaned with Windex™ to remove residual pheromone molecules.

Four-minute mating observations

Observations of mating behavior were made in a clear plastic box partitioned into five cubicles (5.3 x 5.3 x 5.0-cm each). Five, 2-cm-diameter holes were drilled in the box lid over the center of the corresponding cubicles. Organdy material (20 mesh) was placed between the top of the cubicles and the lid to keep moths from escaping. A moth was transferred to a cubicle with the aid of an injector, consisting of a cork plunger inside a 13.0 x 120.0-mm glass tube. The insect was prompted to crawl into the barrel of the injector, which was then placed in one of the holes in the lid of the box. The organdy was gently moved aside and the moth pushed out of the injector with the plunger. Lighting was the same as that described for flight tunnel tests.

Three- to 4-day-old male and 3- to 5-day-old female moths were transferred from holding cartons to 20-ml glass vials. Females were taken from vials and injected into the box, one per cubicle. After females began calling (about 10 min), a male was put into the first cubicle. Timing of several behaviors began immediately. Time prior to sexual activity was the period from introduction of the male into the cubicle to extension of claspers, increased wing movements, and initiation of searching behavior. Time in pursuit of the female was the amount of time that the male spent within 1 cm of the female, trying to mate with her.

Time to successful clasp was the amount of time between introduction of the male and a clasp that resulted in the pair remaining *in copula* for at least one minute.

The number of clasp attempts per male was determined using a hand-held counter. An attempt was counted only if the male was oriented properly vis-a-vis the female, if he attempted to clasp the tip of the female's abdomen, and if his claspers came to within 5 mm of her abdomen. Observations were terminated when a successful clasp was made or at the end of 4 min when another male was put into the next cubicle. If the preceding pair mated during the next 4-min observation period, it was noted, but time to successful clasp was not recorded. For each larval rearing container, five to ten replications were run in one or two partitioned boxes. Males and females tested together were from the same containers.

Two-night mating studies

Two-night mating competitiveness studies were conducted in 11.0-cm-high x 8.5-cm-diameter cylindrical cages made of 3-mm-mesh hardware cloth and capped on the ends with plastic petri plates. Moths were given a 10% sucrose solution for food. Each cage held one female, one "untreated" male, and one "treated" male. In most cases, the "treated" males were merely those from a different rearing container which was set up on the same day as that of the female and the

"untreated" male. The other treatment consisted of adult males exposed to gamma radiation as pupae (see below). Controls consisted of one female and two males from the same container, or one female and two unirradiated males, depending on the test.

To begin a test, 3- to 4-day-old moths were chilled for 15 to 20 min at 4.4° C in a walk-in cold room. During this time, females were taken from 3.8-liter cartons and placed into test cages. Males were marked on the tip of one wing with a red or black felt pen to indicate treatment before being placed in cages with the females. Cages were held in a controlled-environment cabinet and transferred after two nights to a freezer. Females were dissected and the number of spermatophores transferred by males was counted. Both males were also dissected, and the mated status evaluated by the color of the simplex duct near the base of the aedeagus. Dark brown or black indicated the male had not mated, while a clear, cream, or yellowish color meant the male had mated at least once (Snow and Carlysle 1967). Four replications of each treatment and controls were set up.

Treatments

Insects exposed to conditions unlike those in the laboratory rearing program at the USDA were considered to be treated and were used to determine if 1) the testing system was sensitive enough to detect differences between treated

and untreated insects, 2) variables in the rearing program could be manipulated to improve insect quality, and 3) laboratory insects were comparable in quality to wild ones.

Larval diet was modified by changing the concentration of vitamin suspension (see Appendix for composition). Concentrations of 3.3 ml and 12.0 ml were compared to normal amounts of 10.0 ml per 1000 g of diet. Adult diet was modified for other insects by feeding males ad libitum on 10% sucrose solution (standard adult diet for this study), water only, or nothing.

Another treatment consisted of exposing 5-day-old male pupae to between 20 and 35 krad of gamma radiation from a cobalt source. Two groups of 20 - 30 male pupae from the same larval container were placed in separate large test tubes. Tubes from the first group were placed in a circular rack so that each tube was the same distance from the center. The rack was then placed into the irradiator and the source lowered into the center. Dosage was determined by the time of exposure and the distance of the tubes from the source. The second group of pupae served as unirradiated controls. After treatment, both groups were returned to the controlled-environment cabinet with the other insects.

Wild fall armyworm larvae, collected in May and August from corn fields near Alachua, FL, were placed in 3.8-liter cartons containing about 4 cm of sand for pupation. Cartons were covered with 76-mesh organdy and placed outdoors under

a bench that protected them from rainfall and direct sunlight. Larvae were fed daily with corn leaves until pupation. Pupae were harvested when two to three days old and placed in the walk-in chamber with laboratory pupae, where they remained until testing.

Development of Quality Control Charts

An attribute (P) control chart for percent non-usable pupae per container was developed. To establish process capability, the average line on the chart ($\bar{P}$) and the upper (UCL) and lower (LCL) control limits were calculated from the first 20 containers harvested using the following equations:

$$\bar{P} = \frac{\text{total number of non-usable pupae}}{\text{total number of insects}}$$

$$UCL = \bar{P} + 3\sqrt{\bar{P}\ (1.00 - \bar{P})/n}$$

and

$$LCL = \bar{P} - 3\sqrt{\bar{P}\ (1.00 - \bar{P})/n}$$

For example, if the number of non-usable pupae is 528 out of 6892 total pupae, then $\bar{P}$ = 0.077 and the limits are:

$$0.077 \pm 3 \sqrt{\frac{0.077\ (1.000 - 0.077)}{6892}}$$ or, 0.087, and 0.067.

Because the number of insects per container (n) was variable, separate limits were calculated for each container and plotted on a quality control chart (Charbonneau and Webster 1978). P-values and control limits were then calculated for the remaining 16 containers and added to the chart.

Average ($\bar{X}$) and range (R) control charts were prepared for developmental and behavioral traits. Pupal weight will be used to illustrate the development of these charts. Three random samples of five pupae each were selected and weighed for each of eight larval rearing containers. The average and range were determined for each sample and used to calculate the overall average ($\bar{\bar{X}}$) and average range ($\bar{R}$). Range, determined by subtracting the smallest from the largest value, was used in place of standard deviation (s.d.) because it is easier to calculate and because it closely approximates the standard deviation with a sample size of 15 or less. $\bar{\bar{X}}$ was used as the center line for the average chart and the limits were calculated by using $\bar{R}$:

$$UCL = \bar{\bar{X}} + A2\bar{R}$$

and

$$LCL = \bar{\bar{X}} - A2\bar{R}.$$

For example, if n = 5, $\bar{\bar{X}}$ = 218.6 mg, $\bar{R}$ = 66.2 mg, and A2 = 0.577, then the limits are:

$218.6 \pm (0.577)(66.2)$, or 256.8 and 180.4 mg.

A2 is a multiplication factor that varies inversely with sample size (see Juran et al. 1974, Appendix II, p. 39). Therefore, as sample size increases, $A2\bar{R}$, which approximates 3 s.d., will be reduced, and the sensitivity of the chart will be increased. Range charts were developed similarly with:

$$UCL = D4\bar{R}$$

and

$$LCL = D3\bar{R}$$

Using the same example, and D4 = 2.114, then,

$UCL = (2.114)(66.2)$, or 139.9 mg.

D3 and D4 are multiplication factors similar to A2 and are derived from the same chart. The value for D3 when n = 5 is 0.0 so the lower limit for such a control chart is 0. The center line for the R-chart is $\bar{R}$. Control charts for abdominal rotation and WBF were prepared in the same manner as those for pupal weight, except that the sample size for WBF was four instead of five.

An operating characteristics (OC) chart was prepared to complement an $\bar{X}$-chart for pupal weight to determine how sensitive it was for detecting changes in central tendency. An OC chart is based on the z-distribution and represents the probability that the average of a single sample of size n will fall within control limits. Hence, it is an aid in identifying the optimal sample size that will balance the cost of looking for a problem when it doesn't occur (type I error) and the cost of not looking for a problem when it does exist (type II error). The abscissa of the OC chart is the degree of shift from the expected mean of the process ($\bar{\bar{X}} \pm k\,\sigma_{\bar{x}}$, where k is the number of s.d.'s and $\sigma_{\bar{x}}$ = s.d./$\sqrt{n}$; the ordinate is the probability, from 0 to 1, of detecting such shifts. The probability of any given shift being detected was determined by using an expression, k-3, and z-tables for areas under a normal curve. For example, when no shift has occurred, the probability of a sample point falling within control limits is 0.9987 (k=0, 0-3=-3, thus the probability $z > -3 = 0.9987$). But, if the process average shifts to $\bar{\bar{X}}$ + 3 s.d., then the probability of a sample point

falling within control limits is 0.5000 (k=3, 3-3=0; z-value for 0 is 0.50).

Analysis of variance and chi-square tests were used to determine significant differences ($p = 0.05$) and means were separated using Duncan's new multiple range test (DNMRT) with harmonic means calculated when sample sizes were unequal (Chew 1977). Significant values for correlation coefficients and regressions are reported at $p = 0.01$ unless otherwise noted.

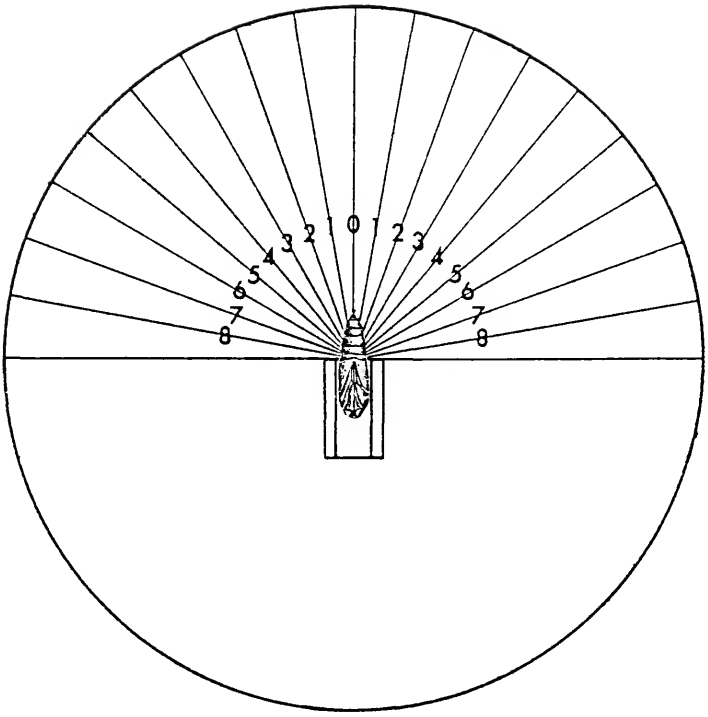


Fig. 1. Device used to determine degree of abdominal rotation for fall armyworm pupae.

Fig. 2. Vacuum-tethering equipment for determining wingbeat frequencies of fall armyworm moths. A=valve used to maintain constant suction pressure; B=valve for turning vacuum on and off; C=illumination source; D=rheostat to control light level; E=pipet for tethering moths; F=stroboscope; G=watchglass for orienting and mounting moths to tether.

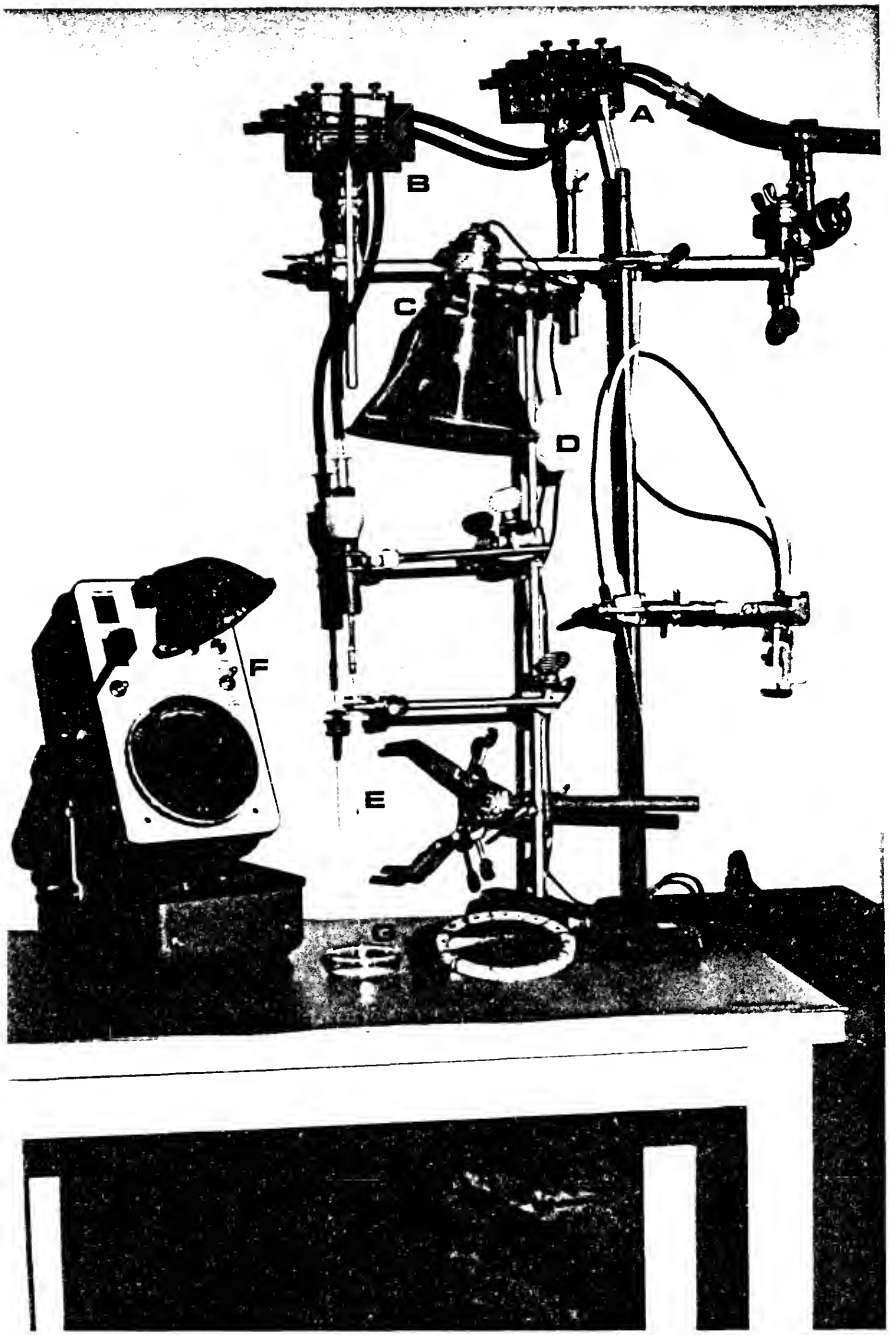
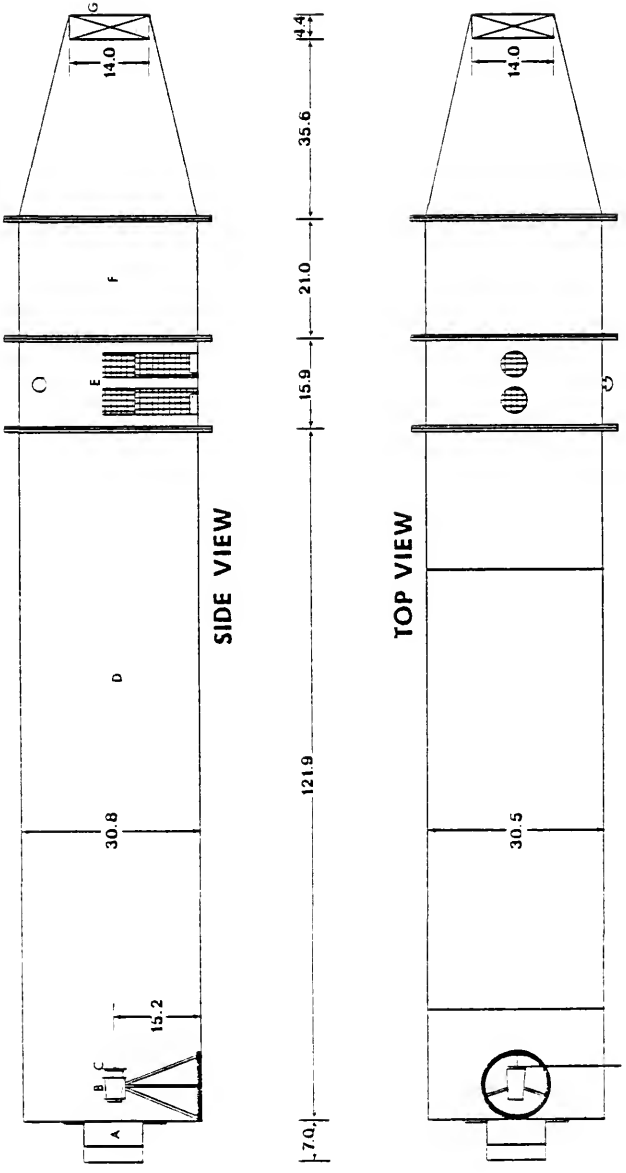

A
B
C
D
E
F
G

Fig. 3. Flight tunnel for observing response of fall armyworm males to a pheromone source. A=exhaust port; B=vial for holding a male; C=starting gate; D=flight tunnel proper; E=cages holding females; F=baffle chamber; G=fan. Measurements are in cm.


SIDE VIEW
TOP VIEW
A
B
C
D
E
F
G
15.2
30.8
121.9
7.0
15.9
21.0
35.6
4.4
14.0
30.5

RESULTS

Occurrence of Dietary Fungal Contamination

Mold, presumably *Aspergillus sp.*, growing on the surface of the artificial diet, occurred in about 35% of all larval rearing containers (n=184). Incidence of contamination was not significantly different among the four container locations or between holding racks, but it varied with set-up day as 38.9, 16.7, 45.0, 25.0, and 47.2% of the containers established on Monday through Friday, respectively, were moldy. The amount of surface area covered by mold in contaminated containers did not show daily differences and averaged 42.8 ± 6.6%.

Insect Density In Larval Rearing Containers

Mean values for densities of normal and abnormal insects per container at the time of pupal harvest differed significantly between 1981, when the rearing containers were first placed into production, and 1982. The total number of insects, percentage of usable pupae, and the percentage not pupating increased from 285 ± 6.9, 88.5 ± 0.4%, and 2.1 ± 0.1%, respectively, in 1981 (n=156) to 338.0 ± 14.0, 91.6 ±

0.5%, and 2.9 ± 0.3% for 1982 (n=72). The upward trend for total number of insects accelerated during the end of the 1982 period (Fig. 4). On the other hand, the proportion of deformed pupae declined from 9.4 ± 0.3% of all insects harvested in 1981 to 5.6 ± 0.3% in 1982. Thus, insect yields increased as experience was gained with the new containers.

The trend in percentage non-usable pupae produced during 1982 was monitored on a control chart (Fig. 5). Limits were calculated individually for each harvest date because of unequal sample sizes. Significantly excessive numbers of non-usable pupae occurred in about 22% of the harvest days (circled points). The average density for these days was 479 ± 41.7 insects per container, much greater than the overall average of 338 ± 14.0. All containers with excessive numbers of non-usable pupae were set up on Monday, Wednesday, or Friday with 63% set up on Friday. Mold occurred in 38% of these containers, not significantly more than the overall average of 35%. On the other hand, those containers yielding significantly more usable pupae (points enclosed in squares) had an average density of 318 ± 52.3 insects per container, and were all set up on Tuesday. None of the containers set up on Tuesday in 1982 had any fungal contamination of the diet.

Total number of insects was correlated with the percentage of usable pupae, with most efficient yields between 250 and 400 insects per container (Fig. 6). The day

containers were set up influenced insect density in 1981 and 1982, and the percentage of usable pupae in 1982, but the daily pattern was not the same as that produced by dietary fungal contamination. Rather, yields from containers set up on Thursdays in 1981 were greater than any other day; Mondays yielded the lowest numbers (Table 1). Variability among containers from all days averaged ± 13.0 but more than doubled to ± 29.5 in 1982 when greatest yields occurred on Fridays, and the average percentage of usable pupae was lowest on Mondays and Fridays (Table 2).

The concentration of vitamin suspension in the artificial diet was strongly correlated with insect yields ($r = -.991$). Between 3.3 and 12.0 ml per 1000 g of diet, yields increased by 50 insects per container for every 1 ml reduction in vitamin suspension. In addition, at 3.3 ml, the number of usable pupae increased by 24% over the highest yield with standard, 10-ml amounts. Standard concentrations of 66 I.U. of vitamin A per gram of diet may have been toxic, as it is known to be in mammals, resulting in degeneration of organs, poor growth, and loss of weight (Maynard et al. 1979).

Characteristics of Immature Insects

Pupal weight

The weights of male pupae were similar for both years and averaged 220.0 ± 1.5 mg (n=3535). However, weights of female pupae declined significantly from 222.3 ± 1.4 mg (n=1660) in 1981 to 216.0 ± 1.8 mg (n=1420) in 1982. The day of container set up influenced pupal weights for males and females in 1981 (Table 1). Containers set up on Monday yielded the heaviest and those from Thursday, the lightest. This corresponded with lowest insect density on Monday and the highest on Thursday; generally, pupal weight was inversely correlated with insect density per container (r= -.416).

Quality control charts were prepared for pupal weights in 1982 of males from container locations B and D, and females from location D (Figs. 7 - 9). R-charts indicated little change in process uniformity over time, the number of points above and below the average range line generally being in balance. Conversely, $\bar{X}$-charts for pupal weight showed downward trends, indicating a general change affecting all samples. Non-randomness in the $\bar{X}$-charts can be identified in the following manner (Juran et al. 1974): Fig. 7 has 12 consecutive points below the average; Fig. 8 has four of five successive points beyond 1 standard deviation; Fig. 9 has two of three successive points beyond

2 standard deviations. Some of the out-of-control points were caused by sampling error, such as in Fig. 7 in which the average weight of the 6th sample was influenced by a relatively large range; the weight of one pupa was much less than average, probably because it was near death. Conversely, other points indicated that production processes were legitimately out of control. For example, values for points 14 and 17 from Fig. 9 showed a real decline in weight because the ranges of these samples were very near normal. This fact, in addition to the obvious downward trend in weights from point 13 on, indicated a significant change was occurring. Comparison of these charts showed that average weights and trends were similar, but the degree of variability differed due to container location and sexes from the same location.

An operating characteristic (OC) curve associated with the average chart for pupal weight from Fig. 9 is given in Fig. 10. This curve shows the probability of not catching a shift in the process average on the first sample taken after the shift has occurred. With successive, independent samples, the probability computed from the OC curve can be used to determine the chance of not observing a shift of a specified amount within two, three, or x samples taken after the shift has occurred (Duncan 1959). For example, if the average pupal weight shifted from 222.8 to 256.5 mg, the probability that the average of a single sample of size five will fall within control limits is about .50, or there is a

50% chance that such a change will go undetected. On the other hand, there is only about a 15% chance of this occurring when the sample size is ten. If the average weight drops only 11 mg, a sample size of five results in a 98% chance of not detecting the change; the larger sample of ten, about a 96% chance. The probability of the change going undetected for five groups of five samples each is $(0.98)^5$ or 90.4% and for five groups of ten samples is $(0.96)^5$ or 81.5%. For most entomological needs, a sample size of five is sensitive enough to detect anomolous trends before harm is done to insect quality. However, at least one variable in a rearing program (e.g. pupal weight) should be monitored regularly with a sample size of ten or more. This referencep variable should be easy to measure and correlate with others that are more difficult to quantify. Sensitivity of the control chart for the reference variable will more quickly alert the manager to developing problems.

Pupal buoyancy

Buoyancy for male pupae was 0.297 ± 0.004 sec and for females 0.318 ± 0.004 sec (n=1420 for both sexes). It was positively correlated with pupal weight (r = .302), making it a possible alternative measurement when taking weights is infeasible or too time consuming. Buoyancy also was negatively correlated with the percentage of diet surface covered by mold (r= -.309).

Abdominal rotation

Abdominal rotation of male pupae averaged 76.0 ± 0.5 degrees and that of the female, 73.9 ± 0.5 degrees (n=1420 for both sexes). For male pupae, rotation correlated negatively with the percentage of diet surface covered with mold (r= -.342) and, for both sexes, it was negatively correlated with density in larval rearing containers (avg. r= -.451).

Quality control charts for male pupae are shown in Fig. 11. Point 10 is out of control in both the range and average charts, indicating that sampling error and not an assignable cause was the reason for the anomoly. However, a slow downward trend in degree of rotation is apparent on the average chart, and it becomes more significant because point 16 is out of control while the corresponding point on the R-chart is near normal. There is some question, however, as to whether point 16 is indeed a warning of declining rotation, especially because the previous five points are so close to average. Sampling error can almost be ruled out because the probablity of selecting a subsample of five pupae with abnormal rotation out of hundreds of normals is negligible. Thus, the probability that a problem does not exist (type II error) is small, and a search for its cause should be initiated. The anomoly may be characteristic of one container or it may be part of a general trend. Production records showed that the container from point 16

did not have abnormally high insect density (364) but that it did have 40% of its diet surface covered with fungal contamination. Verification of a significant downward trend requires further data.

Pupal mortality

The average mortality of insects in the pupal stage was 1.8 ± 0.3% for males (total n=2981, 40 reps) and 1.4 ± 0.3% for females (total n=2667, 40 reps). Increasing diet surface covered with mold increased mortality for both male and female pupae (r= .438 and .385, respectively). Percent pupal mortality for males was fairly constant over time (Fig. 12), but the chart indicated out-of-control values for samples 2 and 17 when excessive mortality was caused by fungal contamination covering 100% of the diet surfaces.

Characteristics of Adult Insects

Eclosion and wing deformity

Of all pupae that appeared to be normal at the time of pupal harvest, 7.3% of the males and 7.8% of the females did not develop into functional adults because of pupal mortality, unsuccessful eclosion, or wing deformities. Of both male and female fall armyworm moths, 1.2 ± 0.3% were unable to separate completely from pupal exuviae (total n=2927 and 2629, respectively, 40 reps. ea.). In males,

unsuccessful eclosion was positively correlated with increased diet surface covered with mold (r= .613), and with reduced buoyancy in water (r= .412). As expected, it was also correlated with increased pupal mortality (r= .565). Wing deformity occurred in 4.3 ± 0.5% of all adult males (total n=2892, 40 reps) and in 5.3 ± 0.8% of females (total n=2596, 40 reps). In females, it was positively correlated with pupal buoyancy (r= .415) and pupal mortality (r= .504). Adult deformity was not influenced by dietary contamination.

Wingbeat frequency

Average wingbeat frequency (WBF) for males was 44.7 ± 0.4 cps (n=358, 32 reps). Males that received no carbohydrate source as adults (n=10) had significantly reduced WBF compared to those that fed *ad libitum* on 10% sucrose solution (41.3 vs. 44.7 cpm, respectively). WBF was negatively correlated with pupal weight (r= -.650).

Control charts for WBF showed consistency over the time period monitored (Fig. 13). Points 10 and 12 on the average chart were very close to the lower limit line and an increase in frequency of low values, without an apparent downward trend, would indicate that all containers or samples were not handled similarly. Investigation of assignable causes would therefore center on factors which affect individual containers such as their location in the larval holding room and number of eggs placed on the diet.

But identification of causes may be difficult because WBF did not correlate with incidence of mold, insect density, abdominal rotation, or pupal buoyancy.

Response of laboratory and wild males to pheromone

Variables of pheromone response for laboratory and wild fall armyworm males are given in Table 3. Nearly all males responded positively to pheromone by extending their claspers inside the release cage. Of those not extending, 83.3% made no attempt to leave the release cage; those that left, did not find the pheromone target. About half of the laboratory males demonstrated anemotactic flight directed to the pheromone source; 83% of the wild males performed this behavior. The remainder of the males either did not fly or flew in a random pattern within the flight tunnel. Only 73% of the laboratory males that arrived at the target did so by flight, while the remainder walked to the source. On the other hand, all wild males reached the target by flight. As a result, the average time required to reach the target was nearly twice as long for laboratory males as it was for wild insects. Only 37% of the laboratory males that arrived did so in less than 30 sec, compared to 71% for wild males. Lab males flew for 20% of the time they were in the tunnel, compared to 46% for wild ones.

Subjective observations also revealed differences between strains. Most laboratory males would leave the

release cage and drop directly to the tunnel floor, 14 cm below. On the floor, they would often hop around in what appeared to be an attempt at flight. Most of those that took flight, however, first climbed to a high surface such as the wall of the tunnel or the platform holding the release cage. Generally, flight was erratic with little hovering and of very short duration. Conversely, wild moths left the release cage and, after an initial flight period, arrived at the source target or landed in the tunnel. These males did not hop and were able to take flight easily from any surface. They flew more precisely, were capable of hovering and slower flight, and had longer periods of sustained flight.

The trend over time for the percentage of laboratory males showing direct flight and the percentage arriving at the target is shown in Fig. 14. Because the variability among samples was so great, and the sample size small (10), the calculated lower control limits would have allowed acceptance of insects that showed no direct flight and that did not arrive at the source target. As a result, subjective limits for percentage showing directed flight (LCLf) and arriving (LCLa) were placed above the values for the four samples with the worst performance for each variable. These limits represent realistic standards which can be used to evaluate strain improvement or deterioration as variables within the rearing program are changed. For example, increasing the percentage showing directed flight

might involve placing insects in mating and oviposition cages that require males to fly to food sources and to females for mating. Moths would be selected for during several generations, and as improvement occured, control limits would be reduced until the colony became stabilized.

The two variables in Fig. 14 have been plotted together because there is a strong correlation between them (r= .719). Consequently, it's expected that males detecting pheromone and orienting to it on the wing, will eventually arrive at the target. This can be evaluated by comparing corresponding points on a given harvest day. For example, on days 9 and 11, the percentage of males that failed to reach the target was higher than the percentage that showed anemotactic flight, indicating that successful orientation during flight did not ensure that the moth would find the pheromone source. This may indicate an inability to sustain anemotactic flight. Conversely, if more males reached the target than showed directed flight, then some moths located the pheromone target by chance, through random flight, or by walking to it, as occurred with half of the males for the 3rd sample.

Four-minute mating observations

Mating behavior of fall armyworm moths was divided into discrete events. Males placed into mating cages containing females did one of two things. They remained motionless with their wings folded rooflike over their abdomens and with their antennae flush against the sides of their bodies or, they immediately assumed an "alert" stance with the costal area of the wings at about a 45 degree angle to the longitudinal axis of the body. Most motionless males became active at some point during the 4-min test period and assumed the "alert" stance. Wings were then vibrated dorso-ventrally through an arc of about ten degrees while the antennae, extended anteriorly, remained motionless. In the next event, males began moving their antennae in a circular pattern. Within 1-2 sec, the antennae were stroked with the epiphysis of the forelegs, followed by extension of the claspers. Wing fanning then increased to an arc of about 80 degrees, thus ending the time period prior to sexual activity. At this point, the male began searching for the female, and upon finding her, he oriented his body parallel to hers, abdomen next to abdomen. The male then extended his claspers and thrust them toward the tip of her abdomen in a copulatory attempt. After clasping the female, the male would turn around and align the length of his body to her longitudinal axis and begin pumping his abdomen, apparently to insert his intromittent organ into the female.

This was accompanied by the male bending his forelegs close to his body and moving his head from side to side. If the pair remained together longer than 1-2 min, the probability of a successful mating was very high. Unsuccessful matings resulted when the male oriented himself improperly with respect to the female, directed his claspers to inappropriate areas, or was rejected by the female. These breakdowns in mating behavior were similar to those described for Heliothis virescens (Teal et al. 1981).

The care and treatment of fall armyworm males directly affected their mating behavior (Table 4). Time to initiation of sexual activity was similar for males fed 10% sucrose solution, unfed, or irradiated as pupae. Seventy-five percent of the fed males became sexually active while only 30% of the unfed and 56% of the irradiated ones did so. Time spent attempting copulation was twice as long for irradiated males than for the other groups. But, during this time, the number of clasp attempts was much higher for fed males than for the other treatments, resulting in 60% of them mating compared to 0% of the unfed and only 11% of those that were irradiated. In a preliminary study, males fed only water mated as successfully (55.6%) as those receiving 10% sucrose solution. Thus, the prevention of dehydration appears to be more important than the uptake of sucrose. The average time to mating was greater for the fed than for the irradiated males and averaged nearly 2 min. However, about 1/2 of the time was consumed before males

began any type of sexual activity. Of the remaining time, the fed males spent only about 30 sec in pursuit of the female. Therefore, the most accurate measure of mating efficiency was the ratio of time in pursuit of the female and the number of mating attempts (Fig. 15).

A trend of increasing variability among samples is apparent at the end of the R-chart. However, the factor responsible is only affecting one insect in each sample because the average chart remains in control, except for observation 13 for which the range was so great that it resulted in an out-of-control point. In the upper half of the $\bar{X}$-chart, points were determined by dividing the number of seconds in pursuit by the number of clasp attempts. In some cases no clasp attempts were made and the time in pursuit was averaged and plotted below the zero line. Thus, the frequency of samples with males actively pursuing females, but not attempting copulation in 4 min, was 33%. The average time of pursuit for this group was 14.9 sec, which is about the same as for those that attempted to clasp. The ratios for unfed and irradiated groups deviated significantly from normal.

The average number of matings remained in control for all samples of untreated laboratory moths (Fig. 16). Because the number of observations differed among harvest dates, an average number was used to determine control limits. This moved the limits slightly further from the center line ($\pm$ .51), reducing the sensitivity of the chart.

This technique is justified if charts are developed from insufficient data. However, equal sample sizes should be used, and when sufficient amounts of data have been collected, new control limits should be calculated.

Two-night mating studies

The average number of spermatophores transferred was 1.7 ± 0.08 (n=176 pairs) with 92.0% of the females mating. The number of matings were in control except for point 10 which represents males that were treated with 20 kr of gamma radiation (Fig. 17). Two-night ratio tests, conducted with irradiated pupae showed that only one treated male (4%) mated, and that the transferred spermatophore was badly deformed. Seventy-six percent of untreated males mated.

TABLE 1. Average number of insects per container and pupal weights for fall armyworms in 1981.

Set-Up Day	Average number of Insects per Container a/b/	Average Pupal Weight (mg)	
		Male	Female
Mon	214.9 ± 9.9 d (29)	225.7 ± 2.2 a (384)	231.5 ± 2.7 a (316)
Tue	254.7 ±14.1 c (32)	220.0 ± 2.7 ab(467)	221.9 ± 3.5 b (333)
Wed	291.1 ±14.7 bc(32)	219.8 ± 1.9 ab(452)	219.2 ± 2.7 b (348)
Thu	349.2 ±11.8 a (31)	213.8 ± 2.2 b (422)	215.7 ± 3.0 b (353)
Fri	310.3 ±14.5 b (32)	216.7 ± 5.0 b (390)	224.7 ± 3.6 ab(310)

a/ Means ± S.E. and (number of observations).
b/ Means in columns not followed by the same letter differ significantly (p=.05) as determined by DNMRT.

TABLE 2. Average number of fall armyworms per container and percentages of usable pupae in 1982.

Set-up Day	Average Number of Insects per Container a/b/	Average Percentage of Usable Pupae
Mon	315.7 ±38.2 b (12)	89.7 ± 1.3 bc(12)
Tue	299.9 ±24.1 b (12)	94.2 ± 0.7 a (12)
Wed	357.8 ±25.5 ab(20)	92.4 ± 0.8 ab(20)
Thu	291.9 ±28.4 b (16)	92.8 ± 0.7 a (16)
Fri	430.1 ±31.2 a (12)	87.8 ± 1.6 c (12)

a/ Means + S.E. and (number of containers).
b/ Means in columns not followed by the same letter differ significantly (p=.05) as determined by DNMRT.

TABLE 3. Behavioral comparisons between laboratory and wild fall armyworm males responding to pheromone in a flight tunnel. a/

Variable	Lab b/	Wild d/
% extending claspers	97.0 ± 0.9	100.0
% not leaving cage	2.9 ± 0.8	0.0
% showing directed flight	55.4 ± 2.5	83.3 ± 7.6
% arriving at target	65.7 ± 2.4	70.8 ± 9.3
% landing inside target	48.3 ± 2.5	70.8 ± 9.3
Avg. time in flight (sec)	8.1 ± 0.5 c/	13.5 ± 1.8
Avg. time to target (sec)	65.4 ± 4.1	38.7 ±10.3

a/ Mean ± S.E.
b/ 40 replications of 10 observations each.
c/ 28 replications of 10 observations each.
d/ 28 observations.

TABLE 4. Comparisons of mating behavior among fed, unfed, and irradiated fall armyworm males. a/

Variable	Fed b/	Unfed c/	Irradiated d/
1 Avg. time before sexual activity (sec)	60.9 ± 7.0	54.3 ±21.7	54.8 ±24.5
2 % extending claspers	75.0 ± 2.6	30.0 ±14.5	56.0 ±16.5
3 Avg. time in persuit of female (sec)	29.2 ± 3.2	18.7 ±10.7	49.3 ±24.6
4 Avg. number of mating attempts	2.7 ± 0.2	0.2 --	0.6 --
5 % males mating	59.8 ± 3.0	0.0	11.0 --
6 Avg. time to mating (sec)	113.6 ± 7.4	0.0	71.0 --

a/ Mean ± S.E.
b/ For variables 1 and 3, n=119 with 13 replications; for 4, n=178 with 32 replications; all others, n=274 with 32 replications. All males fed ad libitum on 10% sucrose solution.
c/ n=10, unreplicated; adults were starved.
d/ n=9, unreplicated; 5-day old pupae were irradiated with 20kr ionizing radiation; adults fed ad libitum on 10% sucrose solution.

Fig. 4. Total number of insects (solid circles) and the percentage of usable pupae (open circles) from fall armyworm larval rearing containers in 1981 and 1982.

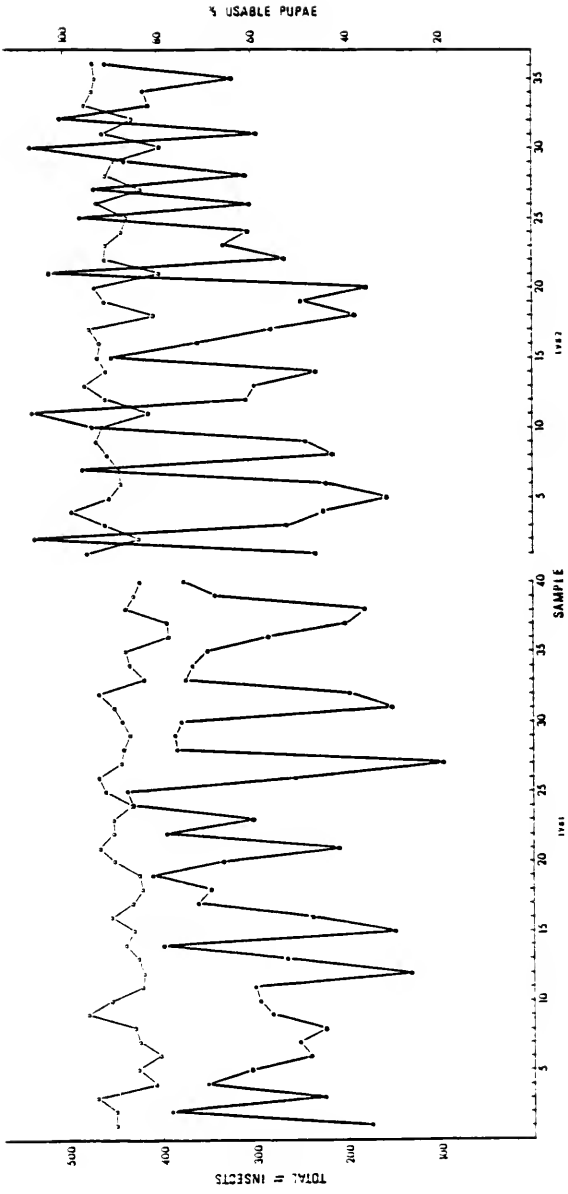

% USABLE PUPAE
100
80
60
40
20
TOTAL # INSECTS
500
400
300
200
100
SAMPLE
1981
1982

Fig. 5. P-chart for the percentage of non-usable fall armyworm pupae from larval rearing containers in 1982. Dashed lines represent control limits derived from samples of unequal size. Circled points exceed upper control limits and points in squares fall below lower limits.

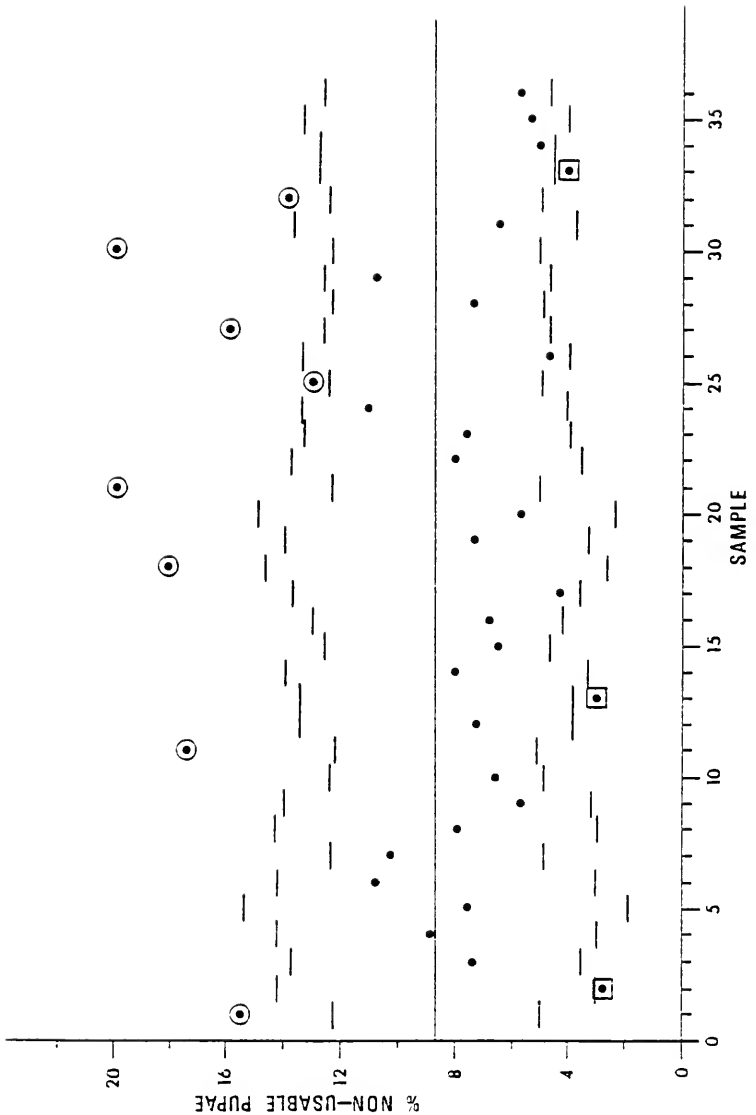

% NON-USABLE PUPAE
0
4
8
12
16
20
0
5
10
15
20
25
30
35
SAMPLE

Fig. 6. The effect of fall armyworm density (D) in larval rearing containers on the percentage of usable (U) pupae. Regression equation is $U = 79.9 + 0.09D - 0.00015D^2$.

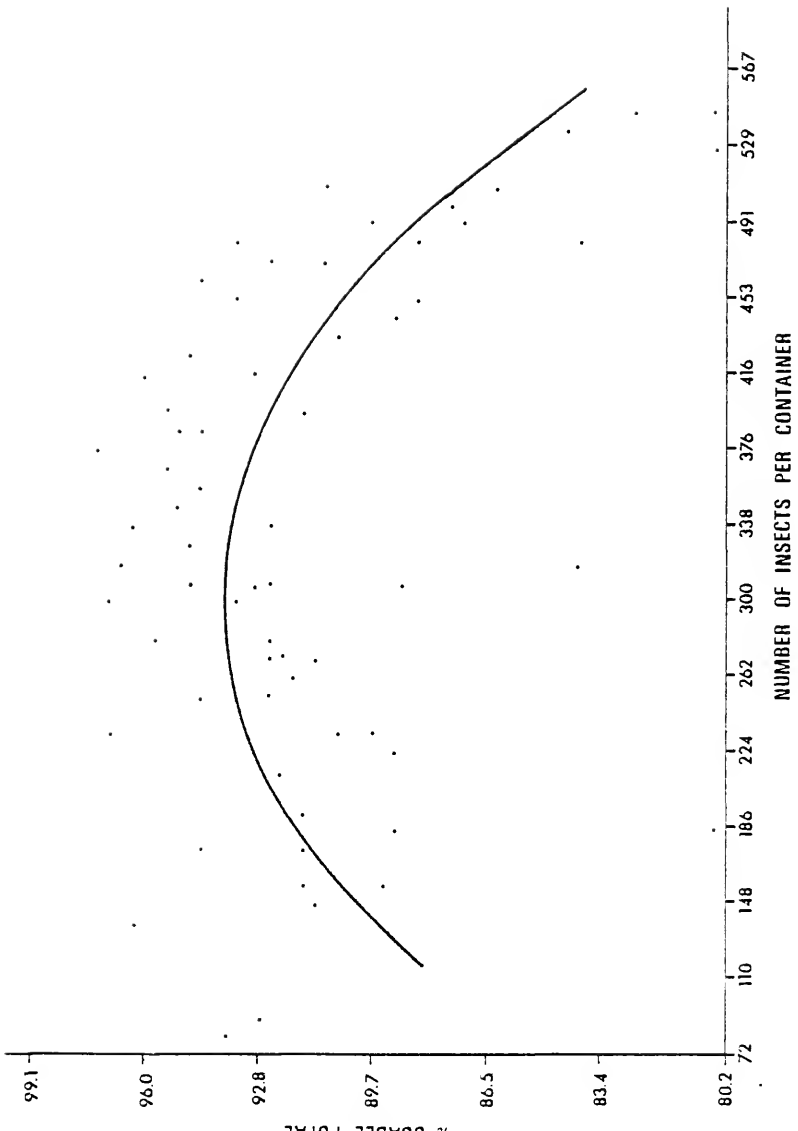

% USABLE PUPAE
99.1
96.0
92.8
89.7
86.5
83.4
80.2
72
110
148
186
224
262
300
338
376
416
453
491
529
567
NUMBER OF INSECTS PER CONTAINER

Fig. 7. Average ($\bar{X}$) and range (R) charts for weights of male fall armyworm pupae from containers at location B on shelves in larval holding room. Heavy dashed lines are upper and lower control limits ($\pm$ 3 s.d.) and other dashed lines are $\pm$ 1 or 2 s.d.; solid lines are chart averages. Sample size=5.

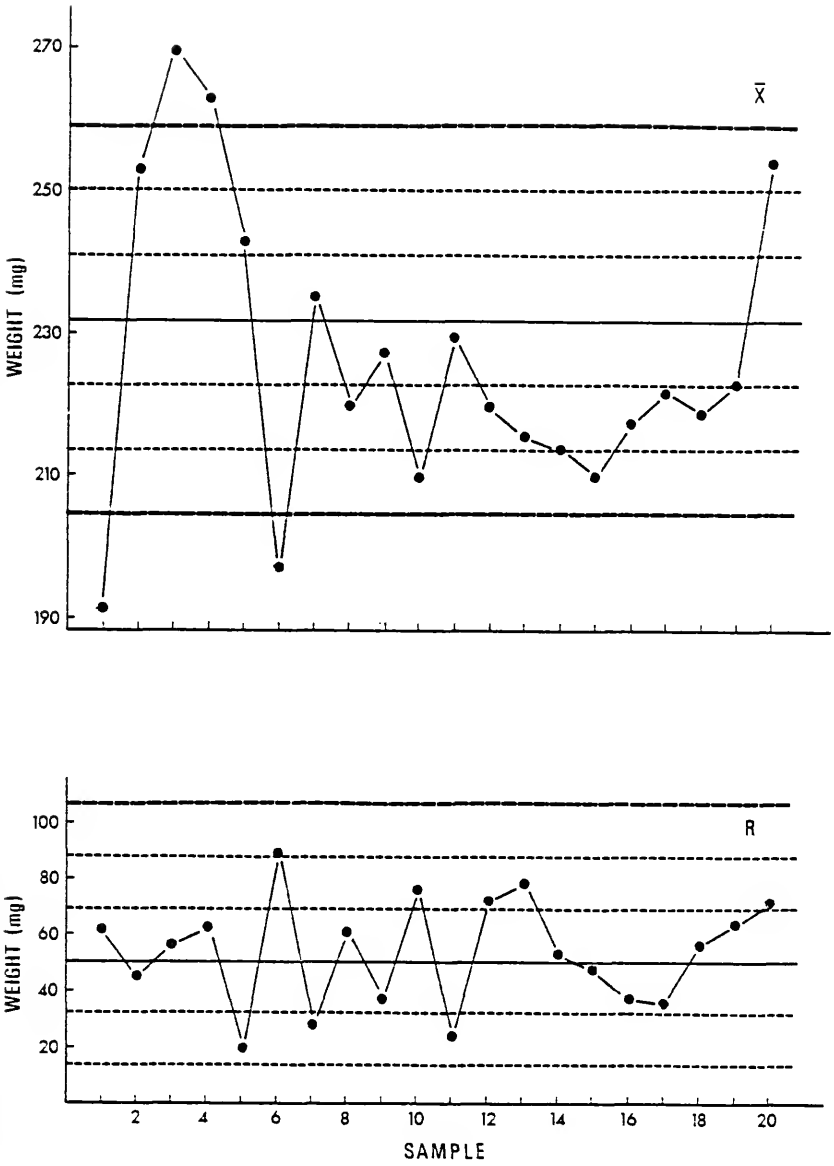

270
250
230
210
190
WEIGHT (mg)
X̄
100
80
60
40
20
WEIGHT (mg)
R
2
4
6
8
10
12
14
16
18
20
SAMPLE

Fig. 8. Average ($\bar{X}$) and range (R) charts for weights of male fall armyworm pupae from containers at location D on shelves in larval holding room. Heavy dashed lines are upper and lower control limits ($\pm$ 3 s.d.) and other dashed lines are $\pm$ 1 or 2 s.d.; solid lines are chart averages. Sample size=5.

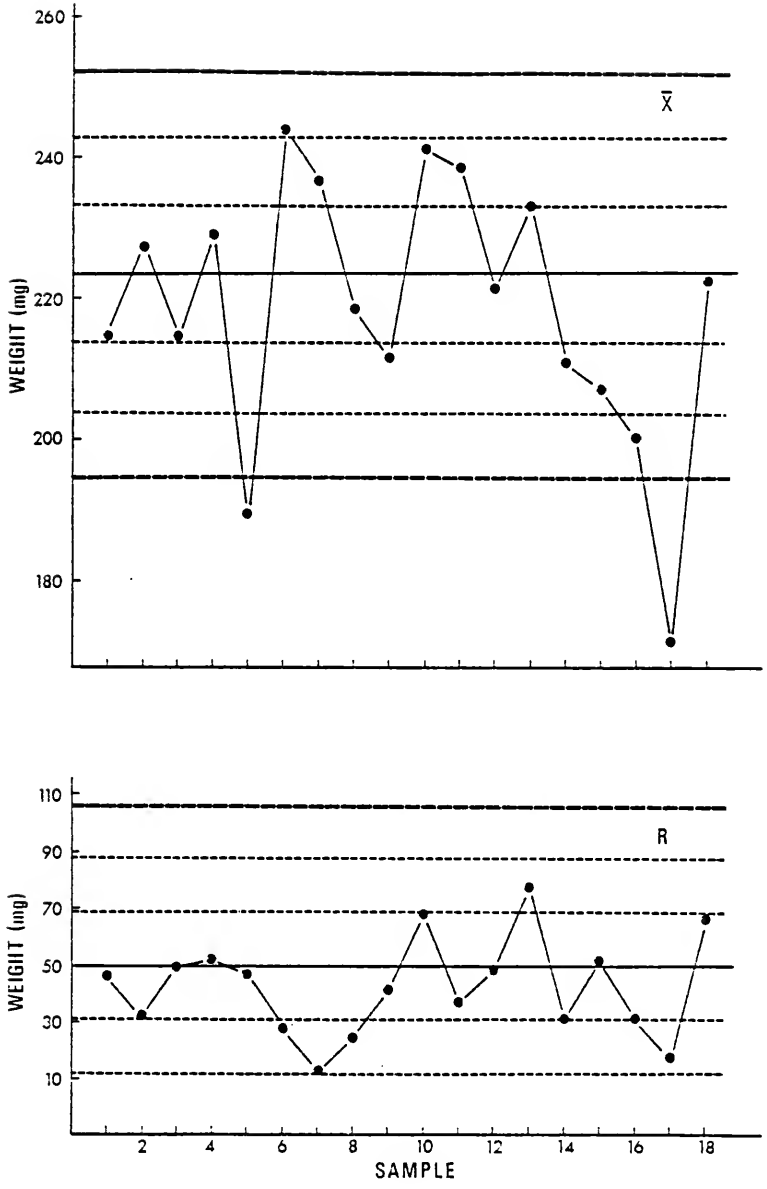

260
240
220
200
180
WEIGIIT (mg)
X̄
110
90
70
50
30
10
WEIGIIT (mg)
R
2
4
6
8
10
12
14
16
18
SAMPLE

Fig. 9. Average ($\bar{X}$) and range (R) charts for weights of female fall armyworm pupae from containers at location D on shelves in larval holding room. Heavy dashed lines are upper and lower control limits ($\pm$ 3 s.d.) and other dashed lines are $\pm$ 1 or 2 s.d.; solid lines are chart averages. Sample size=5.

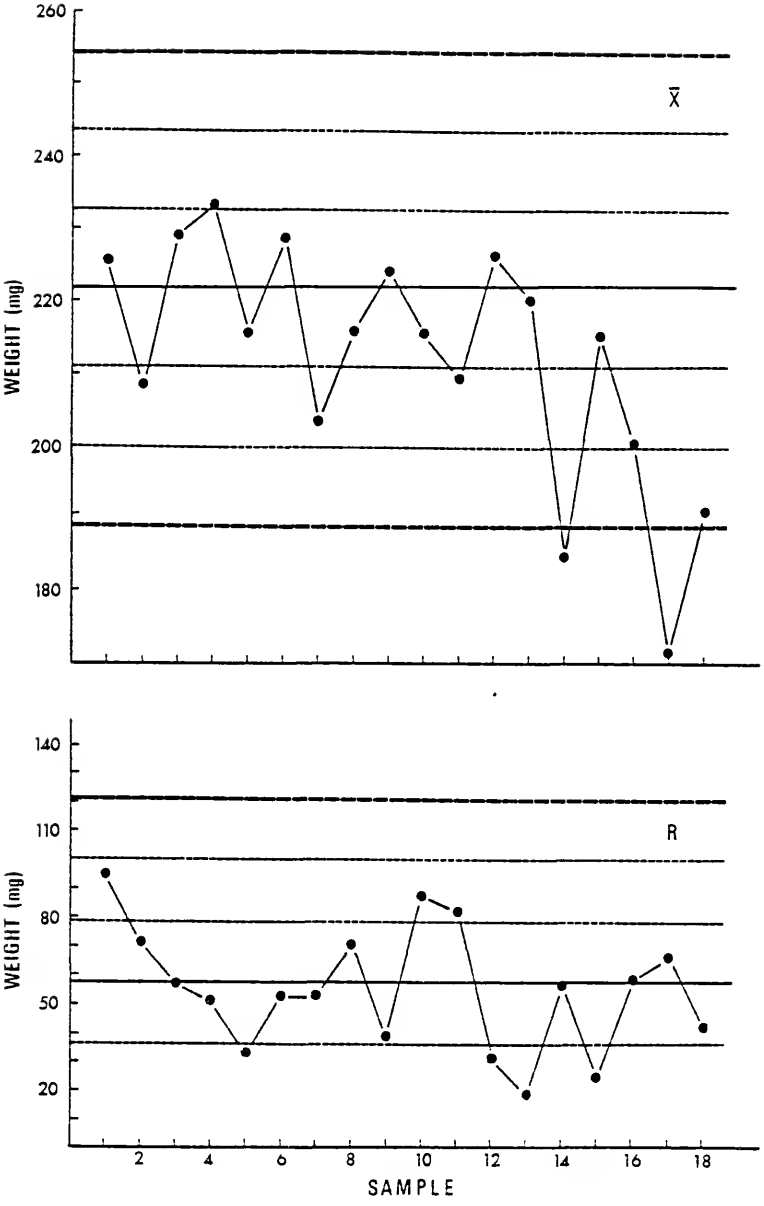
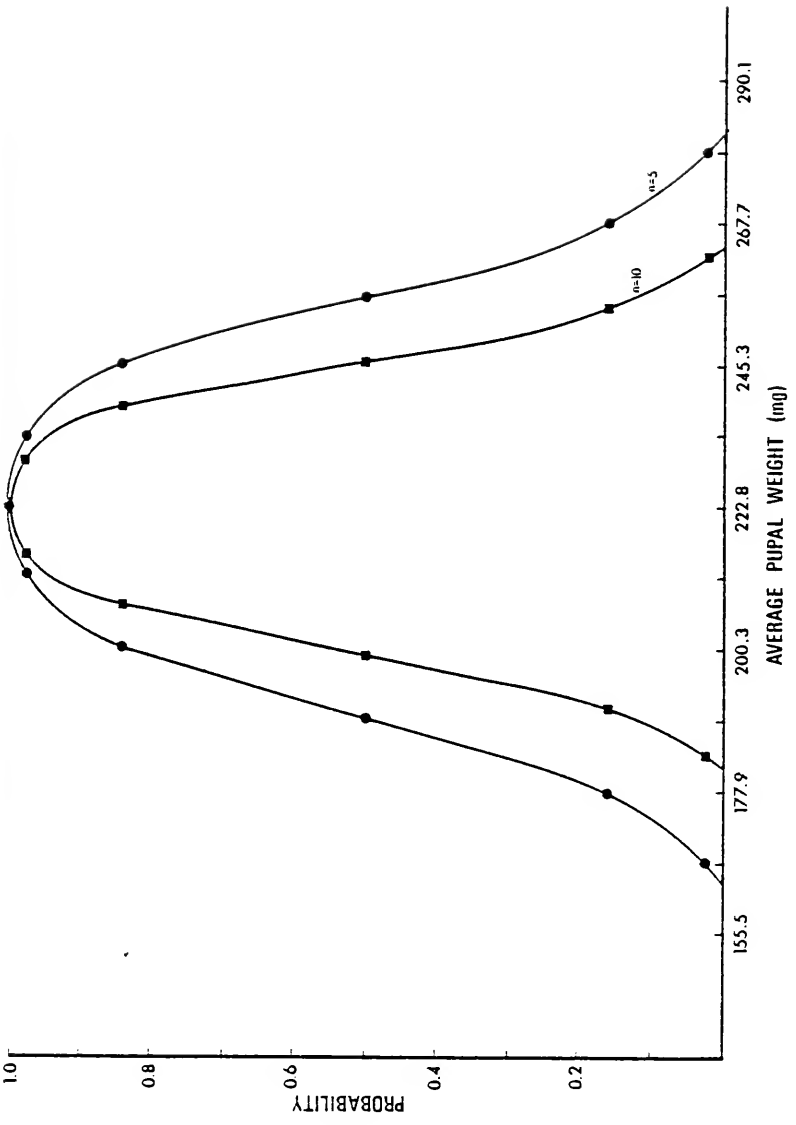

260
240
220
200
180
WEIGHT (mg)
$\bar{X}$
140
110
80
50
20
WEIGHT (mg)
R
2
4
6
8
10
12
14
16
18
SAMPLE

Fig. 10. Operating characteristic curve for weights of fall armyworm pupae from Fig. 9. Ordinate is the probability that the average of a sample of size n will fall within control limits if the process average shifts to a specific value on the abscissa.


1.0
0.8
0.6
0.4
0.2
PROBABILITY
155.5
177.9
200.3
222.8
245.3
267.7
290.1
AVERAGE PUPAL WEIGHT (mg)
n=10
n=5

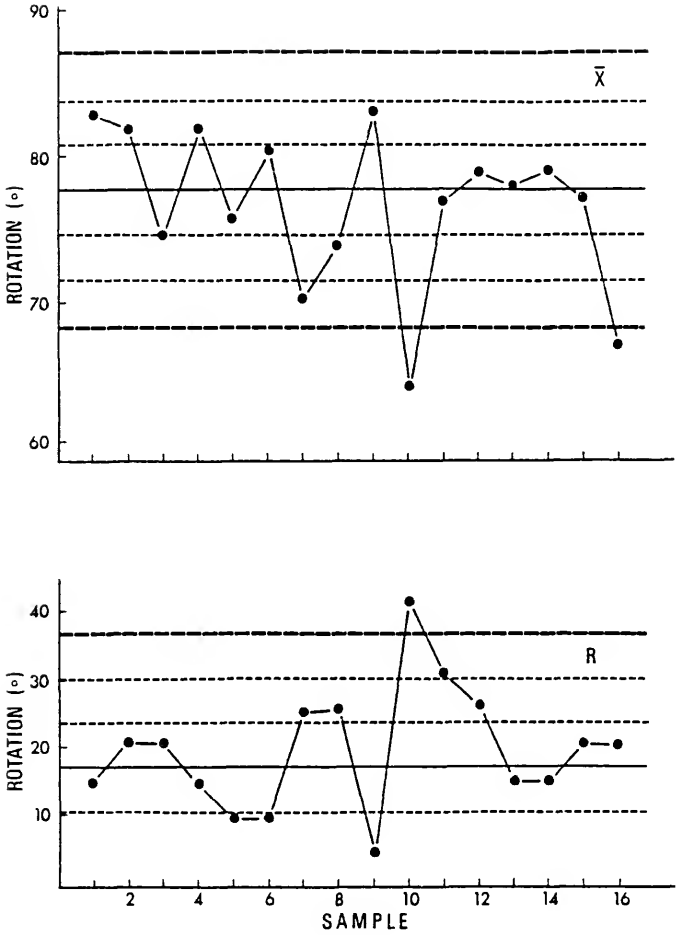
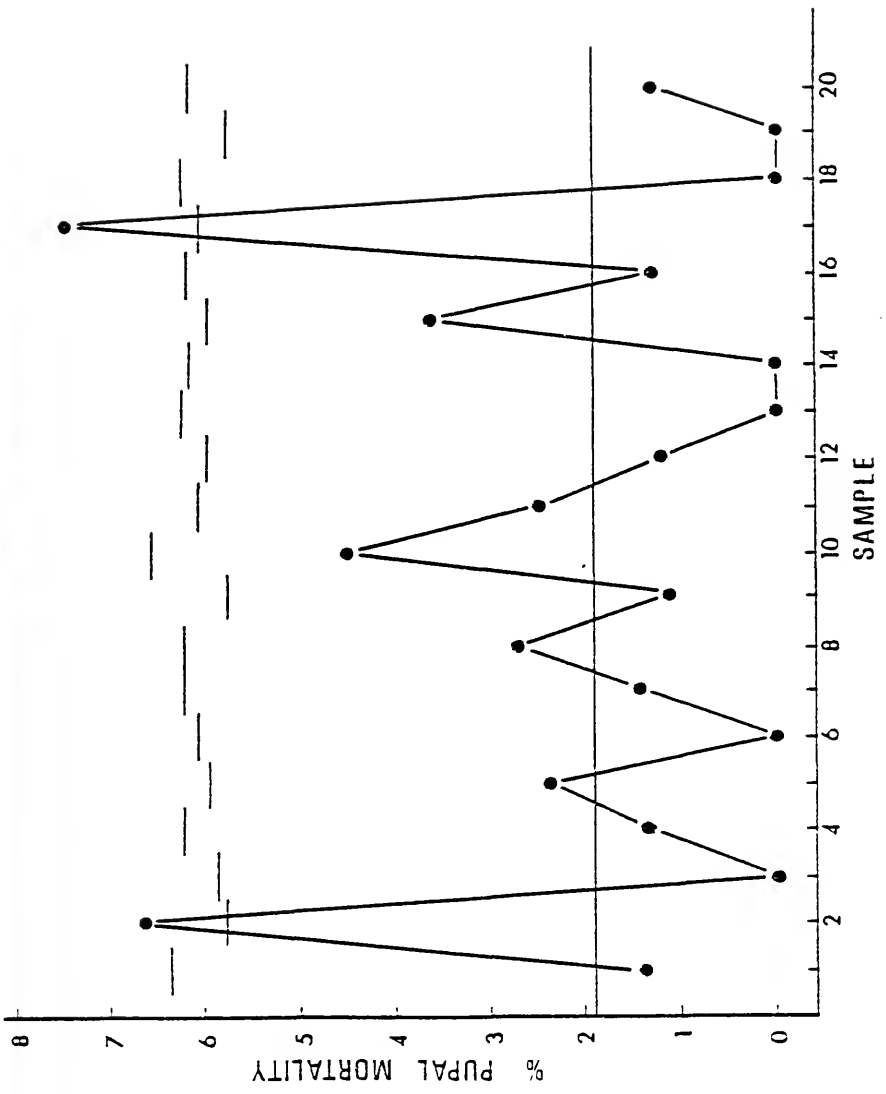


Fig. 11. Average (X) and range (R) charts for abdominal rotation of male fall armyworm pupae from container at location C. Sample size=5.

Fig. 12. P-chart for the percentage of pupal mortality from samples of unequal sizes. Short lines represent upper control limits.


% PUPAL MORTALITY
0
1
2
3
4
5
6
7
8
2
4
6
8
10
12
14
16
18
20
SAMPLE

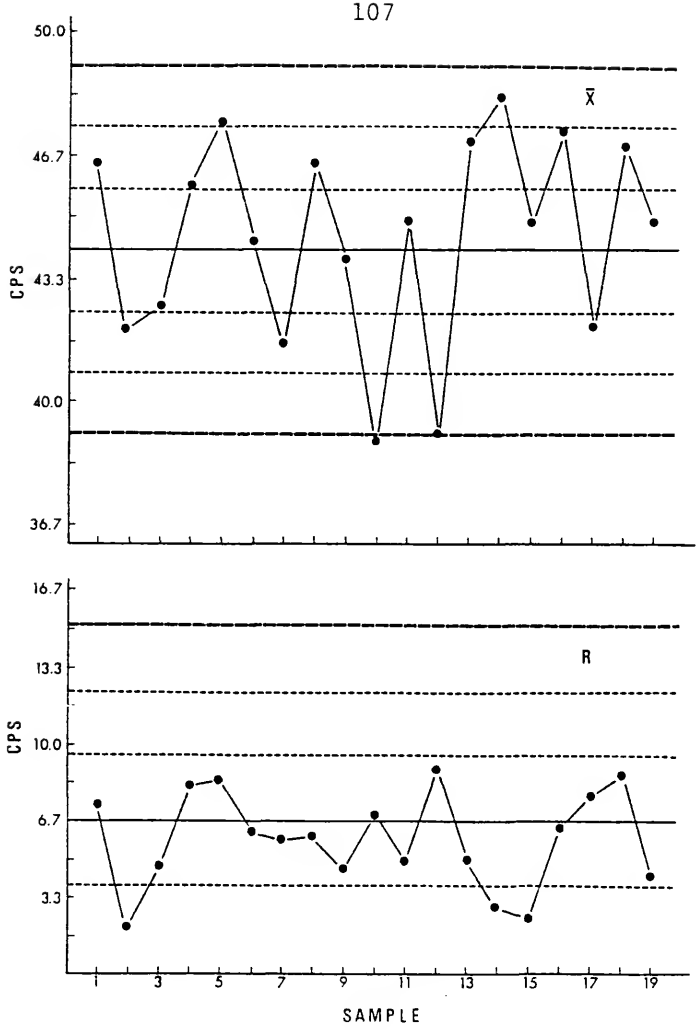


Fig. 13. Average (X) and range (R) charts for wingbeat frequencies of male fall armyworm moths. Ordinate is cycles per second. Sample size=4.

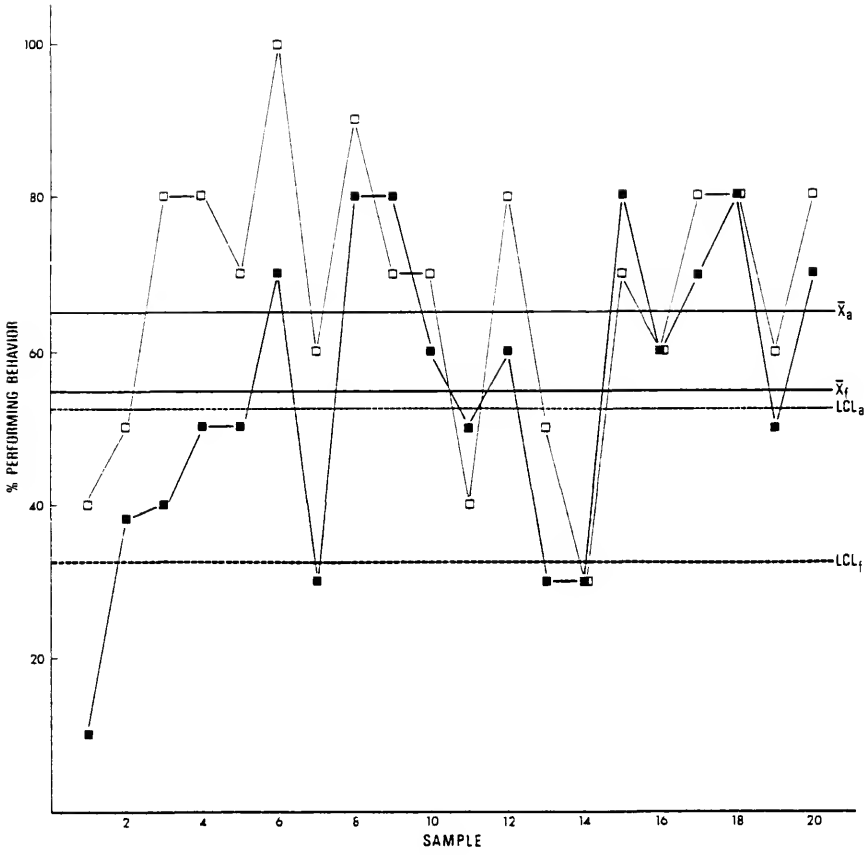
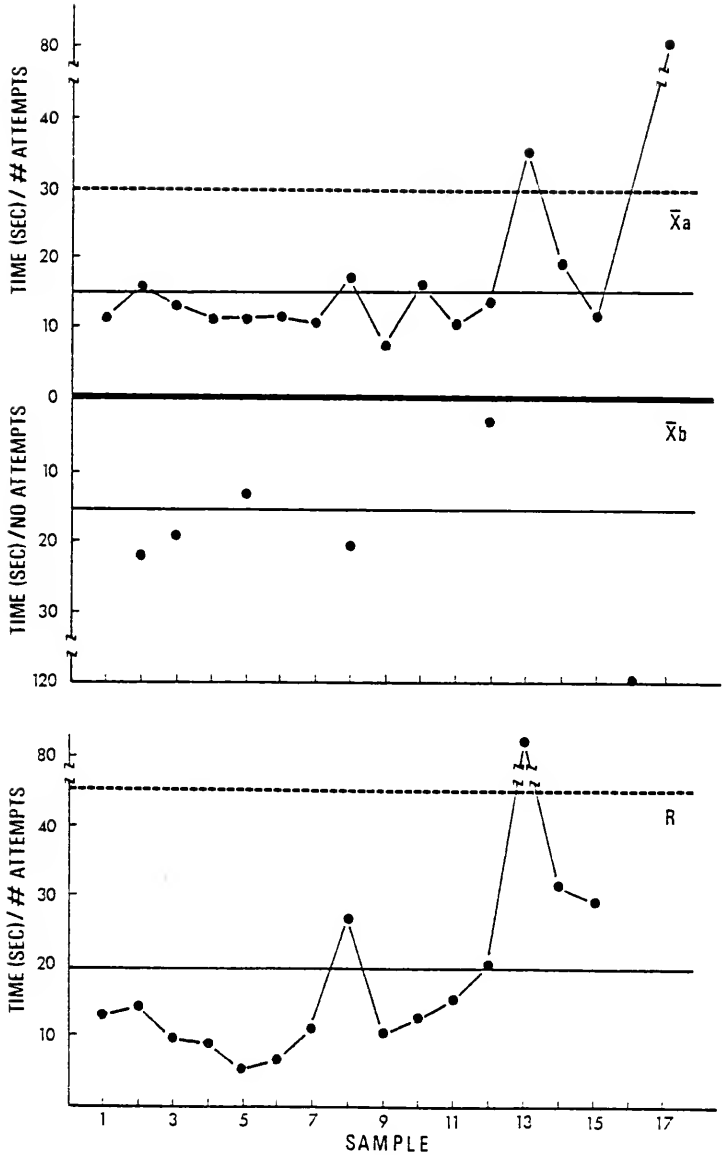


Fig. 14. P-chart for the percentage of male fall armyworm moths showing anemotactic flight (solid squares) and those arriving at the pheromone source (open squares). X and LCL are the average percentages and lower control limits for flight (f) and arrival (a), respectively. Sample size=10 for each behavior.

Fig. 15. Average ($\bar{X}$) and range (R) charts of mating efficiency (seconds in pursuit of the female divided by the number of mating attempts) for fall armyworm adults. $\bar{X}b$ is the average time in pursuit for males not attempting copulation. Sample 16 on the $\bar{X}b$-chart represents males unfed as adults, and sample 17 on the $\bar{X}a$-chart represents males from irradiated pupae. Dashed lines are upper control limits; single solid lines are chart averages. Sample size=4.


TIME (SEC)/ # ATTEMPTS
80
40
30
20
10
0
$\bar{X}a$
TIME (SEC)/NO ATTEMPTS
10
20
30
120
$\bar{X}b$
TIME (SEC)/ # ATTEMPTS
80
40
30
20
10
R
1
3
5
7
9
11
13
15
17
SAMPLE

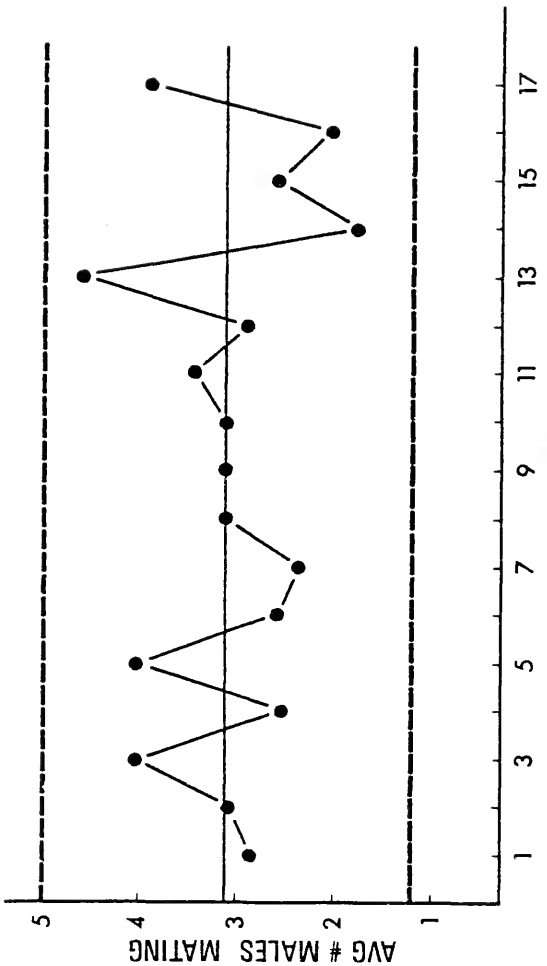


Fig. 16. Average number of males mating during four-minute observations. Dashed lines are upper and lower control limits. Sample size=4 and each sample represents the number of matings per five pair of moths.

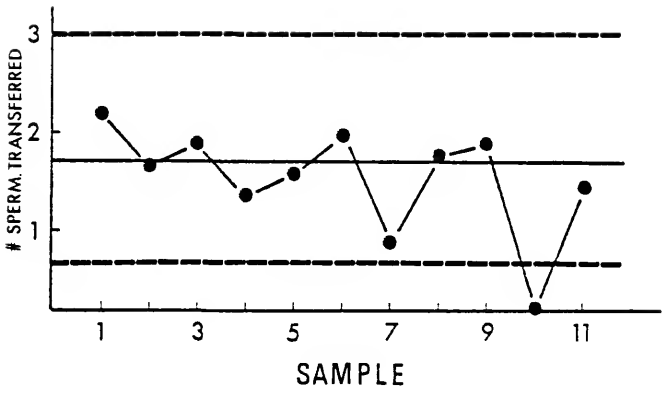


Fig. 17. Average (X) chart for the number of spermatophores transferred during two-night mating studies. Dashed lines are upper and lower control limits representing average values for sample sizes between 12 and 16.

DISCUSSION

The major findings of this study are 1) the quality control tests outlined are sensitive enough to detect changes in processes by measuring insect characteristics, to aid in the identification of variables that affect insect quality, and to demonstrate relative quality of the laboratory strain; 2) dietary fungal contamination occurs frequently in larval rearing containers and adversely affects insect quality; 3) pupal traits such as weight, abdominal rotation, and mortality are sensitive indicators of changes that occur in production; 4) these traits may be indicative of adult quality; 5) adult behavior is highly variable and is not as sensitive as pupal traits in detecting changes in insect quality; 6) flight performance of laboratory fall armyworm moths is inferior to that of wild standards tested in the laboratory; 7) analysis of flight and mating behavior is time consuming but is vital for precise definition of problems with insect quality; 8) process control charting is an effective means to identify significant changes or trends in insect traits; and 9) a quality assessment program separate from the production system does nothing in itself to improve insect quality.

Fungal contamination may cause spoilage of diet,

deterioration of nutrients, and accumulation of physiological products that may be poisonous to insects (Sikorowski in press). In this study, mold occurred in one of every three containers randomly selected for testing and covered, on average, nearly half of the surface of the diet. Mold was one of the most important variables affecting insect quality, as it increased pupal mortality and the incidence of incompletely eclosed adults. Although the presence of mold reduced abdominal rotation and buoyancy, no direct effects on adult behavior were observed. However, mold was weakly corrrelated ($p = .06$) with increased time to pheromone target in the flight tunnel.

Fungal contamination may originate from many sources within the rearing program. The fact that most incidences of mold occurred on Monday, Wednesday, and Friday indicates a possible association with daily activity schedules in the insectary. On these days, procedures such as egg and pupal harvest, surface sterilization, and placement of eggs on diet were required for six species; on Tuesday and Thursday, only three species were processed. The increased work load on these three days may have resulted in insufficient time to adequately clean reusable containers or to effectively complete other procedures. Also, it required more movement of personnel within the insectary, thereby increasing the probability of spreading contaminants.

Another possible hypothesis for mold growth is that while spores are omnipresent (e.g. in dietary ingredients,

on egg sheets, and in the air), their germination depends on proper environmental conditions. For example, unsanitized egg sheets that fell from container lids to the diet surface invariably produced mold growth originating from the point of contact. I encountered a similar problem with codling moth when surface-sterilized egg sheets fell onto larval diet. Presumably, no mold spores were present on these treated sheets, but they inhibited moisture exchange and created stagnant pockets that optimized the environment for the germination of spores. Contamination produced in this manner is easily prevented by securing the sheets to container surfaces. Many moldy fall armyworm containers, however, did not have fallen egg sheets, so other factors must have been responsible for altering conditions. Free moisture within the container was influenced by variable volumes of artificial diet which acted as a moisture source, and by the amount and rate of air flow across the top of the container. In preliminary studies, no relationship existed between variability of diet (thickness and texture) and mold occurrence. The location of excessively dry diet within larval containers at the time of pupal harvest was correlated with areas of increased air flow within the room. Mold tended to grow where diet was more moist. If moister diet was more suitable for larvae, they could have accumulated in those areas, thereby adding respiratory moisture and compounding the problem.

Dietary fungal contamination is a difficult problem to

solve because a balance must be achieved between an adequately moist nutrient source and a diet that progressively dries to permit efficient pupal harvest. To achieve uniform dessication and reduced mold, air flow should be reduced around containers by covering racks with plastic or other transluscent material. Relative humidity within the room can then be reduced so that controlled drying of the diet can take place. Potential sources of contamination should be identified. Using a general microbial medium such as potato dextrose agar (PDA), dietary ingredients, equipment, walls, egg sheets, employees, and air within the rearing facility can be monitored (Sikorowski 1975).

Process control charting indicated frequent out-of-control points for the percentage of non-usable pupae in containers that had very high larval densities. These containers were set up on Monday, Wednesday, and Friday, implicating activity schedules as the causal factor. Mold incidence, also abnormally high on these days, did not correlate with the number of non-usable pupae. The chart also indicated a $\pm$ 8% variation in percentage of non-usable pupae among containers. From a production control standpoint, this represents too much uncertainty for predicting yields.

One determinant of density is the number of eggs used to set up larval containers. In this study, these numbers were not quantified because of difficulty in separating egg

masses from ovipositional substrates and in separating individual eggs. Some techniques employing chemical treatment (McMillian and Wiseman 1972) or water pressure (Gross et al. 1981) have been proposed. However, excessive egg mortality occurred with both procedures. Another approach to egg quantification is allowing the eggs to hatch, mixing neonates with ground corn cob to the desired concentration, and distributing premeasured amounts to rearing containers. This method has been effective for other lepidopterous larvae (Davis and Oswalt 1979) but it increased the potential for larval mortality, dietary contamination, and application time. Another technique, similar to the vacuum tethering device, is used to pick up individual neonate larvae which are counted and delivered directly to the diet surface. This technique causes little or no damage to larvae and 300 can be transferred in about four minutes.

Controlling insect density is important for the production of quality insects. It was shown that high density is strongly correlated with reduced pupal weight and abdominal rotation. Indirect effects on adult behavior may have resulted from changes in pupae that are affected by density. Further, the potential for disease incidence increased in crowded rearing containers. High densities may cause stress resulting in physiological changes that make the insects more susceptible to infection (Steinhaus 1958) or they may increase the frequency of contacts among insects

leading to greater transmission of pathogens (Tanada 1965).

The reduction in yields at low densities may have resulted from a factor that caused increased mortality of eggs. Fifty-three percent of the containers with less than 250 insects had eggs that were fertilized but unhatched at the time of pupal harvest, three weeks after oviposition; those with more than 250 insects had almost no unhatched eggs. Whatever affected egg mortality may also have adversely influenced developing larvae and resulted in an increased number of pupal deformities.

The search for assignable causes for downward trends in $\bar{X}$-charts for pupal weight was based on identifying similar or opposite trends in variables that are correlated with it, such as insect density. Such a correlation provided a tangible means to explain the trend and relate it to specific factors in the rearing environment that can be observed, recorded, and changed. These include the number of eggs, the amount and quality of artificial diet, contamination, environmental conditions, and personnel activities. Evaluation of production records and consultation with employees provides further insight into changes in insect quality and helps establish a course of action to reverse the trend.

There were profound differences in the behavior of laboratory and wild insects in the flight tunnel. While most laboratory males extended their claspers in the presence of pheromone, many were unable to fly, orient, and

arrive at the source. Presumably, these males would be unable to find females in nature. Change in such an important behavior is probably related to confinement of adults in small mating and oviposition cages, resulting in selection for moths with reduced flight propensity and perhaps capability. Thus, the performance of wild males in the pheromone tunnel is a more realistic standard of required flight performance than that of internal standards based on the ability of laboratory moths. As such, it could be identified on a control chart as the desired value for flight behavior of laboratory insects and used as a guideline for improving quality of the colony.

Based on internal standards, irradiation and deficiencies in adult diet adversely affected mating behavior and competitiveness of laboratory males. However, it also appears that the mating ability of the untreated laboratory males was suboptimal. Some of them extended claspers and began what appeared to be searching behavior, but no attempt to copulate was ever made. In other cases, males carelessly directed abdominal thrusts at the females. The few wild males were much more able to orient themselves to females and to precisely direct their abdominal thrusts.

Process control charting was shown to be a sensitive tool for identifying changes in insects during colonization. Charting of important variables should be on-going in any rearing program whether or not realistic standards are available. Charts are initially used to establish process

consistency; only then can standards be realistically applied. Sampling frequency depends on the stage of colonization, the type of tests performed, and the amount of time and resources committed to quality control. Ideally, samples should be taken more frequently whenever wild stock is introduced or infused into an existing culture, or when production processes have been modified. Deleterious changes can occur much more quickly during these periods than when the colony has become stabilized. The sampling strategy used in this study for monitoring traits of immatures two to three times per 5-day work week is barely adequate for early stages of colonization. These variables were easy to measure, required no sophisticated equipment, and used such small sample sizes that testing could be done every day. Adult behavior was analyzed about two times per week which is sufficient for newly colonized populations. Samples must be taken from rational subgroups (Bicking and Gryna 1974) that have essentially been treated the same. In my work, the rational subgroup was the larval rearing container. Data from different containers were pooled only after it became known that insect quality was unaffected by container location.

Once stability has been achieved in a colony, say over a three to five generation period, the sampling frequency may be reduced to once per week for more complex behavioral tests, such as flight performance and mating observations. More sensitive indicators of colony status, such as pupal

weights, abdominal rotation, mortality, and wing deformities should be evaluated at least two to three times per 5-day work week. Time requirements for this schedule using tests described in this report, is about six hours per week based on values presented in Table 5. To ensure adequate evaluation of the effect of set up days on insect quality, a regular, 4-week sampling regimen should be initiated. Two samples should be taken during the first and third weeks and and three samples during the second and fourth weeks. The sampling frequency for the second 4-week period is reversed to ensure identification of cyclical trends in quality that have the same frequency as the sampling plan. If changes are made in rearing procedures, or if a problem in the program is suspected, testing should be more concentrated. Analysis of adult behavior should be conducted on the same day when other tests are performed so correlations can be made between them.

A Model Quality Control Program

The most complete and effective quality control program is one which is thoroughly integrated into the production system. Thus, the model program will be presented in this manner. It assumes that insects will be reared on artificial diet and yields will be about 50,000 pupae per day. The model is based on release of males sterilized by ionizing radiation, a component of an area-wide control

program for fall armyworm suggested by Knipling (1980). The model will be developed around the production facility, efficient procedures that allow natural insect behavior, quality testing, process control charting, and a feedback system. It emphasizes laboratory testing of insect quality and must not be extrapolated to performance of released insects unless complementary assays are conducted in the field.

The rearing facility

The rearing facility provides the environment in which insect production occurs (Fig. 18). The physical layout influences the flow of materials and products, the organization of supplies and equipment, and the isolation of sanitary areas (Fisher in press, a). The design separates areas of high contamination potential (e.g. pupal harvest room) from those that must remain sanitary (e.g. diet preparation). The spread of contaminants between these areas is minimized by restricting access, enforcing regulations, and installing equipment such as a double-door steam autoclave (SA) and ultraviolet light passthroughs (PT).

Generally, the flow of materials and products is unidirectional to prevent backflow of contaminants. Egg sheets from the perpetual, or nucleus colony are collected in Room 5 and sanitized to remove microbial contaminants in

Room 14. Then the sheets are placed in sterile flasks that are capped, labelled, and transferred to the Set-Up Room 16 where they are incubated until hatch. Neonate larvae are transferred to rearing containers, most of which are taken to the Mass-Production Area 17 where they remain until pupation. Pupal harvest, radiation treatment, and packaging occur in Room 18. The other containers are taken to Larval Holding Room 6 for the nucleus colony. Upon pupation, these containers are transferred to Harvest Room 11 where yields are determined. Pupae are placed in a sterile container and passed into Room 14 where they are disinfected. Used, plastic cell units are washed, soaked for 1 hr in sodium hypochlorite, and transferred to Storage Room 12. Heat-stable items, such as larval containers and adult cages, are washed, sterilized in the double-door autoclave (SA, Room 11), and stored in Room 12. Clean cell units are filled with artificial diet in Room 13, taken to Set-Up Room 16, placed in containers, and implanted with larvae.

Periodic evaluation of microbial load is made to ensure sanitation of the facility and proper functioning of insectary equipment, such as the diet mixer and air-conditioning-supply ducts. Swabs taken from walls, floors, and equipment are plated on general media (PDA and trypticase soy agar) for fungi and bacteria. Results are used to indicate the level of contamination and effectiveness of sanitary procedures. The effectiveness of passthroughs is monitored by exposing open agar plates

inside the unit for 15 min before and after illumination of UV lights. Indicator tape that changes color upon exposure to sterilizing conditions is used in the autoclave with every load. Experience with microbial assessment will indicate the most significant sources of contamination, which can then be monitiored more intensively and charted.

Procedures and quality testing

The nucleus colony is derived from 400-500 wild individuals collected in southern Florida during March or April. Succeeding generations are infused with 50-100 insects a year if data show genetic decline of the population. In either case, late-instar larvae are brought into the laboratory through Quarantine Room 1 and are reared under conditions that maximize survival. Under fluctuating temperature and RH, larvae are fed natural food until pupation. Then, two pairs of male and female moths are kept in each cage placed over 5-cm-diameter flower pots containing bermuda grass that serves as an oviposition site and food for larvae of the next generation. Containers with second instar larvae are placed in a refrigerator for eight hours to stress insects and activate transovarially-transmitted pathogens like cytoplasmic polyhedrosis virus and microsporidia that may be present (Allen 1981). Containers with diseased insects are discarded. Third and 4th instar larvae are removed from the

grass and placed individually in cups containing artificial diet to which surface-sterilized grass clippings have been added. Dead or slowly developing larvae are discarded. Random samples of larvae are selected for determining microbial load using procedures discussed by Allen (1981). After eclosion, adults are placed into large oviposition cages in Quarantine Room 2 fitted this time with paper toweling for an oviposition surface. Egg clumps are collected, sanitized, placed in a sterile flask, and held in an incubator in Room 2. After hatch, about 2000 neonates are placed in a larval rearing container that is set up in addition to those of the nucleus colony. Wild larvae are held separately in Quarantine Room 2 so that, if disease is detected, they can be disposed of without endangering the nucleus colony. After harvest, the percentage of usable pupae is determined and measurements are taken on pupal weight and abdominal rotation. Wild insects with significantly abnormal values are not used for infusing the nucleus colony.

Wild and colonized pupae are mixed, separated by sex, and placed in emergence cages. Begining of adult age is recorded when 25% of the pupae in each cage eclose. On the second night post-eclosion, each cage of females is placed into a 1.6-m flight tunnel (Fig. 19) in Room 3. Between the hours 2000 and 0200, a black light is illuminated automatically at the end of the tunnel opposite the females. Able insects fly to the light and are captured in another

cage. A conical-shaped trap keeps females from walking into the cage or leaving it once they have been captured. The next morning, determinations are made of females that did not leave the eclosion cage, that left but were not able to fly into the capture cage (number remaining in the tunnel), that had deformed wings, that were unsuccessfully eclosed, and that died as pupae. The females that were captured at the light are used as reproductives to perpetuate the nucleus colony. That night, three days post-eclosion, five of these females are caged and placed at one end of a 1.8-meter tunnel in Room 4. Males, still in their eclosion cages, are placed in the opposite end (Fig. 20). A small fan blows air across the female cage, down the tunnel, across the male cage, and into flexible hose to be exhausted outside of the facility. The fan operates between 2000 and 0200 to attract males to the pheromone-laden breeze. Safegards, similar to those for the female tunnel, keep non-flying males from entering and captured moths from leaving the cage. These tests ensure that the reproductive stock will consist of individuals that are active during the peak reproductive period, can detect and orient to black light, wind, and pheromone, and are able to fly at least 1.8 m, and maneuver while flying.

The next morning, four days post-eclosion, determinations are made of male pupal mortality, wing deformities, and the number arriving at the capture cage. These data are entered onto process control charts. In

addition, 20 male and 20 female reproductives are distributed into each of five mating and oviposition cages. These 30.5-cm^3 cages are constructed of clear, autoclavable polycarbonate plastic (Fig. 21). Two rectangular holes, cut in the bottom and covered with 0.6-cm-mesh galvanized hardware cloth, are used for scale collection. A small platform on the bottom provides a resting area for moths during photophase. Four sugar-water feeders, located in the walls, have reservoirs that can be filled from outside the cage without disturbing the moths. The top has a 20.3 x 22.0-cm opening covered by paper toweling attached to a metal frame by spring clips that serves as an oviposition substrate. At the time of egg harvest, moths clinging to the toweling are gently tapped off, the frame is removed, and a new one is quickly put in place.

Egg sheets are harvested daily and taken into Room 14 where they are sanitized. Before and after treatment, small, random samples are set aside to determine fertility. Samples consist of medium-sized egg masses on 20-mm disks of paper toweling cut by a cork-hole borer. Disks are labeled, placed in glass vials with tight fitting lids, transferred to an incubator set at about 27° C and 70% RH, and checked daily for egg hatch (five replications per treatment). Larvae are counted and aspirated off so they do not feed on remaining eggs. After all hatch has occurred, the total number of larvae is calculated and the number of unhatched eggs, fertile and unfertile, is determined by using a

dissecting microscope. Percent fertility is calculated and entered on a process control chart.

Eggs for mass production are placed in sterile Erlenmeyer flasks with screw-on lids modified to accept an inverted graduated centrifuge tube. Flasks are put into an incubator so that the only light visible to larvae is through the tubes. Neonate larvae are positively phototactic and negatively geotactic so they crawl up the flask and into the tip of the centrifuge tube where they congregate. The tube is removed and the volume of larvae estimated. It is then placed in the bottom of the larval rearing containers.

These containers represent a new approach to fall armyworm mass production and are designed to allow natural larval behavior, simplify set up and pupal harvest, save space, and reduce production costs (Fig. 22). The container consists of a 36.0 x 39.4 x 36.0-cm-high clear polycarbonate box with parallel structures to support polystyrene cell units previously filled with artificial diet. The bottom of the box is covered with about 4 cm of sterile vermiculite and the top is covered with a tight-fitting lid that contains screen-covered holes for air exchange. Neonates crawl out of the centrifuge tube to the top of the cell unit and begin feeding. Prepupae are positively geotactic and therefore move down to the container floor and pupate in the vermiculite. At harvest, the cell units are removed and pupae are sifted from the vermiculite and frass. The number

of insects per container must be determined empirically, but an estimated capacity of 3000 to 5000 pupae is possible for mass production. A yield of 4000 in the new container represents a 700% and 200% reduction in space requirements compared to 30-ml cups and the form-fill-seal containers, respectively. A significant savings in labor, materials, and equipment would also be realized. For the nucleus colony, only one container with 2000 larvae is set up each day.

Ten, second or third instar larvae from the nucleus colony are randomly selected from each container and taken into the quality control laboratory. The larvae are placed in petri plates on diet surface-treated with an aqueous solution of insecticide, such as methomyl, at a concentration to kill 50% of the larvae in 24 hours. Mortality scores are charted and excessive values indicate the possible presence of stressors such as overcrowding or disease, or of a basic change in physiology. Other larvae are electorphoretically analysed for enzyme production that has been shown to change during colonization. These enzymes include hexokinase, esterases, and alpha glycerophosphate dehydrogenase. Differences in enzyme concentration over time may mean a change in gene frequency or a change in gene expression. Remaining larvae are allowed to pupate at which time they are harvested in Room 11.

After harvest in mass-production, 150 pupae are randomly selected twice weekly from pre- and post-irradiated

populations. Both groups are taken into the quality control laboratory, sexed, weighed, measured for abdominal rotation, and placed into individual cartons according to sex and treatment. Date of 25% eclosion is noted and, on the third day post-eclosion, thirty males, from each group and a comparable sample of the same age from the nucleus colony, are chilled to about 7° C. The moths from each group are marked with a different colored felt pen at the tip of one wing, pooled into one container, removed from the cold room, and placed in the 3.4-m flight tunnel in the Quality Control Room (F.T. in Fig. 18). Ten females from the nucleus colony are placed at the opposite end of the tunnel behind a trap that captures responding males in a manner similar to that described for the nucleus colony. The next morning, the number of males from each treatment that arrived at the female trap is determined by observing colors on wing tips.

In a similar manner, five more males from the same sample groups are chilled, marked, and placed together in small 3.8-liter cartons along with 15 females from the nucleus colony. The test is replicated five times. Cartons are then taken to a holding cabinet where they remain for 48 hours before the moths are frozen and dissected to evaluate mating propensity and competitiveness. Mating status in males is determined by the color of the simplex (Snow and Carlysle 1967), and in females by the presence or absence of spermatophores. The test is replicated five times and data are recorded on a control chart.

The tests and observations proposed for a model quality control program include five tests that monitor pupal mortality, adult morphology, flight performance, and orientation to habitat (Table 6). Quality of the nucleus colony can be assessed daily because monitoring techniques are an integral part of the rearing procedure and, thus, require little extra time. Fecundity should also be determined daily but pupal weights and abdominal rotation, sensitive indicators of change in rearing processes, are measured every two to three days for the nucleus colony, irradiated, and unirradiated mass-produced insects. Egg fertility, larval challenge with insecticide, mating competitiveness, and response to pheromone are evaluated every three to four days.

Direct observations of flight performance and mating behavior, as described in the Methods section, are conducted when necessary to evaluate specific changes in essential behavior patterns. Most results are plotted on an $\bar{X}$, R, or P-chart, while other data are filed for future reference. Each month, pupal weight and abdominal rotation are conducted at least twice for each day containers are set up and for all other tests, at least once (Table 7). This sampling strategy is adequate to show developing trends, effects of set-up day on insect quality, and cyclical variation that may be present.

Because process control charts do not identify what factors cause changes in insect quality, it is imperative to

keep records on variables associated with rearing procedures. These data are essential to the feedback system, and without them, quality control is limited to acceptance/rejection sampling.

Procedural data are collected frequently, sometimes continuously, and may be displayed on charts similar to those used for process control. Variables such as temperature and relative humidity, recorded continuously by hygrothermograph, strip-chart recorder, or data logger, should be closely monitored during changes in season and when the air flow balance has been altered within the facility, as when filters are replaced.

Diet production should be monitored every day. The amount per container can be estimated volumetrically or by weight and moisture content can be determined by sampling with a tensiometer near each corner and at the center of a cell unit. Other variables that should be recorded include modification of procedures or dietary ingredients, changes in ingredient suppliers, preparation dates for solutions such as KOH and vitamin mixes, the highest temperature to which the diet was exposed during preparation, and the time period between preparation and use. Subjective observations about diet characteristics, such as color, texture, and gelling time, should be made by experienced individuals. A check list of ingredients, their amounts, and preparation techniques should be readily available. The employee that prepares the diet should initial the data sheets.

Variables that may indirectly affect insect quality should be monitored. These include the date when filters are changed, equipment is serviced, and electrical or pneumatic service is interrupted as in a power outage. It must be noted when alarm limits are exceeded, visitors enter the building, especially the sanitary areas, and when employees go on sick leave or vacation. Treatment of eggs and pupae with surface sterilants can be damaging to insects. Records are made when new bottles of sterilant are put into use and when dilute solutions are prepared. Employees performing these procedures should also initial data sheets.

Feedback system

Monitoring insects and procedural variables will provide the data required to maintain and improve product quality. However, once an out-of-control point or trend is observed on the process control charts, systematic evaluation of the problem is necessary before action can be taken. In most cases where only one or two points in $\bar{X}$- or R-charts exceed control limits over a long period, the cause is usually quite apparent and can often be remedied by discussion with the responsible employee or by modifying or servicing a particular piece of equipment. On the other hand, increasing frequencies of out-of-control points should be viewed with concern as this indicates a recurring

problem. Perhaps the most difficult problems to identify and solve are those characterized by upward or downward trends on control charts. This means that the insects or processes are changing so slowly that it would be imperceptible without a process control chart. Such changes can occur during colonization, when selection eliminates certain genotypes or, when a new procedure is instituted, it is done cautiously and precisely until its impact on the system can be evaluated. Once this has been done, the conscientousness of the employee may decline, with estimates replacing precise quantification, and result in a drift of a measurable insect trait.

Examples of the systematic approach to problem solving in an insect production program are given in Figs. 23 and 24. These dichotomously-branching flow charts are similar to taxonomic keys in which specific questions are asked to guide the user to the most appropriate solution. The more procedural data available, the more sensitive and accurate the chart. In Fig. 23, a significantly reduced yield of usable pupae is noted. Records are checked to determine if any type of contamination occurred. If a disease is suspected, larval or pupal samples are sent to a pathologist for confirmation. In the meantime, employees are alerted and the search for other evidence of disease begins. If disease occurs in containers set up on different days, a general emergency is called and employees are to proceed with caution, being aware of any potential sources of

contamination. Further isolation of certain areas may be required and special procedures are invoked to monitor and maintain sanitary requirements. Conversely, if no disease is suspected, other variables are examined until the appropriate solution is identified. Frequently, an assignable cause is never found.

This example illustrates the need for procedural data to supplement control charting. At the time of pupal harvest, the employee did not know that yields of usable pupae were out of control. Thus, the worker had no reason to pay particular attention to other characteristics of the container or its insects. By the time the results were plotted and the problem identified, few relevant details could be recalled. Routine monitoring and recording of pertinent data prove invaluable at this time.

Fig. 24 is a similar flow chart that is based on a decline in flight ability of pre-treated, mass-produced (MP) males. This illustration makes use of an internal standard, or males from the nucleus colony. If both colonies show the same decline, the cause lies in the nucleus colony or in some variable the two colonies have in common, such as artificial diet. If, on the other hand, the decline is noted only in the untreated, MP males, then the cause is related to a factor unique to that part of the production system, such as larval density or temperature.

TABLE 5. Time required to conduct procedures and tests described in this study.

Procedure or Test	Time (min)
1. PUPAL CHARACTERISTICS	
a. Count normal, abnormal, and non-pupae (per container)	5
b. Sex 150 pupae	.10
c. Weight (20 pupae)	.10
d. Abdominal rotation (20 pupae)	.10
e. Pupal buoyancy (20 pupae)	.10
TOTAL	.55
2. ECLOSION CHARACTERISTICS (60 - 80 INSECTS)	
a. Determine emergence	5
b. Determine mortality, eclosion, deformities	.5
TOTAL	.10
3. WINGBEAT FREQUENCY (10 INSECTS)	
a. Test set up	3
b. Test	.30
c. Cleanup	.5
TOTAL	.38
4. RESPONSE TO PHEROMONE (10 MALES)	
a. Test set up	5
b. Time before females begin to call	.10
c. Test	.40
d. Cleanup	.6
TOTAL	.61
5. FOUR-MINUTE MATING OBSERVATIONS (10 PAIR)	
a. Test set up	5
b. Time before females begin to call	.10
c.$Observation	.50
d. Cleanup	.3
TOTAL	.68
6. TWO-NIGHT MATING COMPETITIVENESS STUDY (10 REPS)	
a. Test set up, mark insects	.30
b. Dissect females	.10
c. Dissect males	.15
TOTAL	.55
GRAND TOTAL	291

TABLE 6. Summary of tests and observations for model quality control program.

Test or Observation	Insects Tested a/	Freq. b/	Charting $\bar{X}$	Charting R	Charting p	File Only
1 Egg fertility	N	4			x	
2 Insecticide challenge	N	4			x	
3 Pupal weight	N,UI,I	6	x	x		
4 Abdominal rotation	N,UI,I	6	x	x		
5 Pupal mortality c/	N	Daily			x	
	UI,I	4			x	
6 Adult eclosion c/	N	Daily				x
	UI,I	4				x
7 Wing deformities c/	N	Daily			x	
	UI,I	4			x	
8 Response to light (females)	N	Daily				
a % arriving at light trap					x	
b % remaining in flight tunnel						x
c % remaining in eclosion cage						x
9 Response to pheromone (male)	N	Daily				
a % arriving at pheromone trap					x	
b % remaining in flight tunnel						x
c % remaining in eclosion cage						x

TABLE 6 -- continued

10 Mating competitiveness	N,UI,I	4	x	
11 Response to pheromone	N,UI,I	4		x

a/ N=nucleus colony; UI=unirradiated, I=irradiated mass-produced insects.
b/ Frequency of test or observations per 14 day period based on a 7-day work week.
c/ For the N colony, these observations are made for females in test 8, and for males in test 9.

TABLE 7. Sampling strategy for quality control testing and observations. a/

Day	Week 1	Week 2	Week 3	Week 4
M	1,2N 3,4N,UI,I 5,6,7UI,I 10,11N,UI,I		3,4N,UI,I	
T		1,2N 3,4N,UI,I 5,6,7UI,I 10,11N,UI,I		3,4N,UI,I
W	3,4N,UI,I		1,2N 3,4N,UI,I 5,6,7UI,I 10,11N,UI,I	
T		1,2N 3,4N,UI,I 5,6,7UI,I 10,11N,UI,I		3,4N,UI,I
F	1,2N 3,4N,UI,I, 5,6,7UI,I 10,11N,UI,I		3,4N,UI,I	
S		3,4N,UI,I		1,2N 3,4N,UI,I 5,6,7UI,I 10,11N,UI,I
S	3,4N,UI,I		1,2N 3,4N,UI,I 5,6,7UI,I 10,11N,UI,I	

a/ Numbers refer to test or observation in TABLE 6. N=nucleus colony, UI=unirradiated, I=irradiated mass-produced pupae. 5,6,7,8,9N sampled daily.

Fig. 18. Facility for rearing 50,000 fall armyworm pupae per day. Solid arrows show direction of insect movement and open arrows of containers only. FT=flight tunnel; PT=passthrough equipped with ultraviolet lights; SA=double-door steBm autoclave.

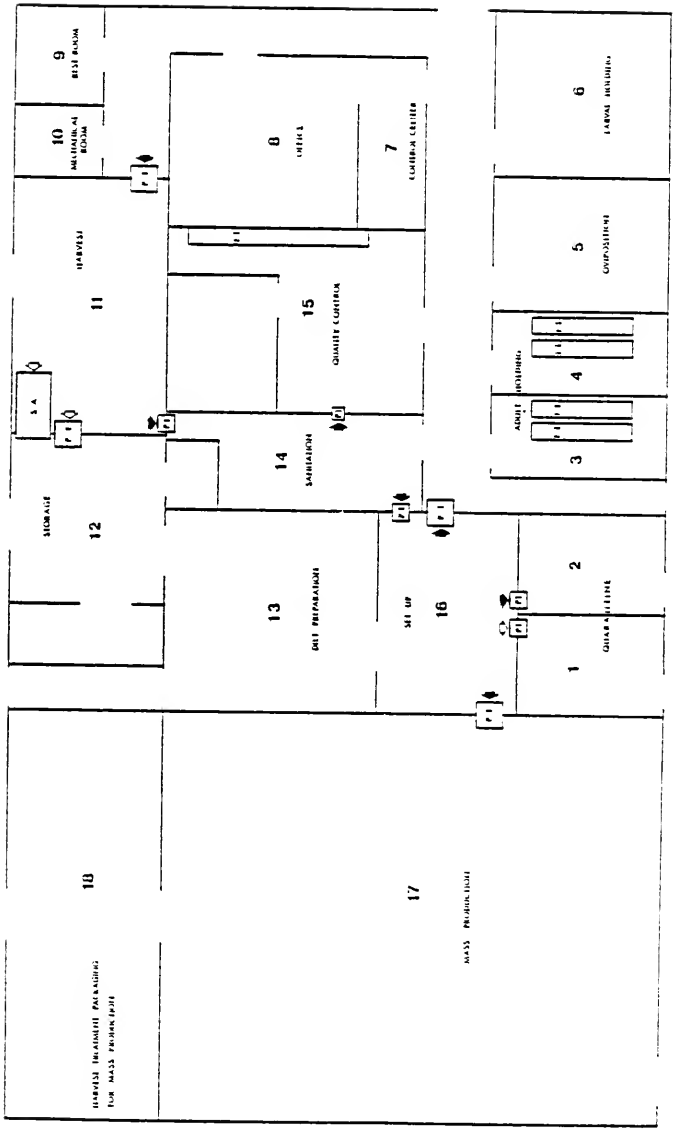

9
10
8
7
6
5
4
3
11
15
14
12
13
16
2
1
18
17

Fig. 19. Flight tunnel for collecting female fall armyworm moths for use in the nucleus colony. A=eclosion cage; B=antechamber; C=0.3 x 1.8-m flight tunnel; D=cone trap that allows only flying moths to enter capture cage; E=capture cage; F=scale collection shelf; G=black light.

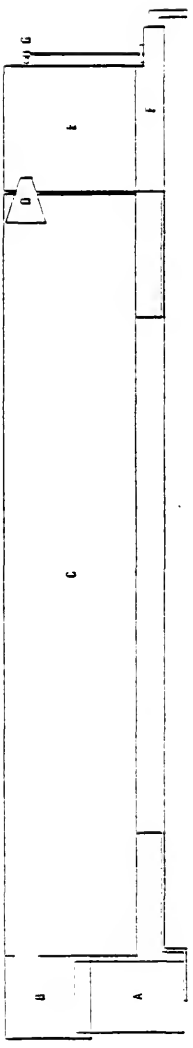

G
F
E
D
C
B
A

Fig. 20. Flight tunnel for collecting male fall armyworm moths for use in nucleus colony. A=exhaust port; B=antechamber; C=emergence cage for male pupae; D=0.3 x 1.8-m flight tunnel; E=cone trap to allow only flying moths to enter capture cage; F=capture cage; G=fan and cage for females.

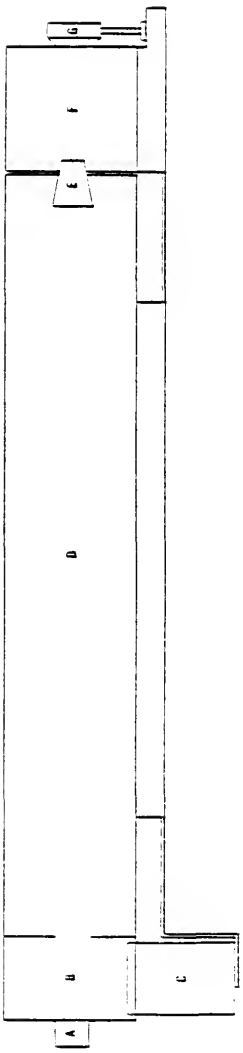

G
F
E
D
B
C
A

Fig. 21. A 30.5-cm^3 mating and oviposition cage for fall armyworm adults. A=screen-covered opening in floor for scale collection; B=resting area for moths during photophase; C=externally-mounted feeders for liquid diet; D=support frame for oviposition substrate; E=hanging substrate to increase oviposition area; F=feeder tube that encourages moths to hover while feeding.

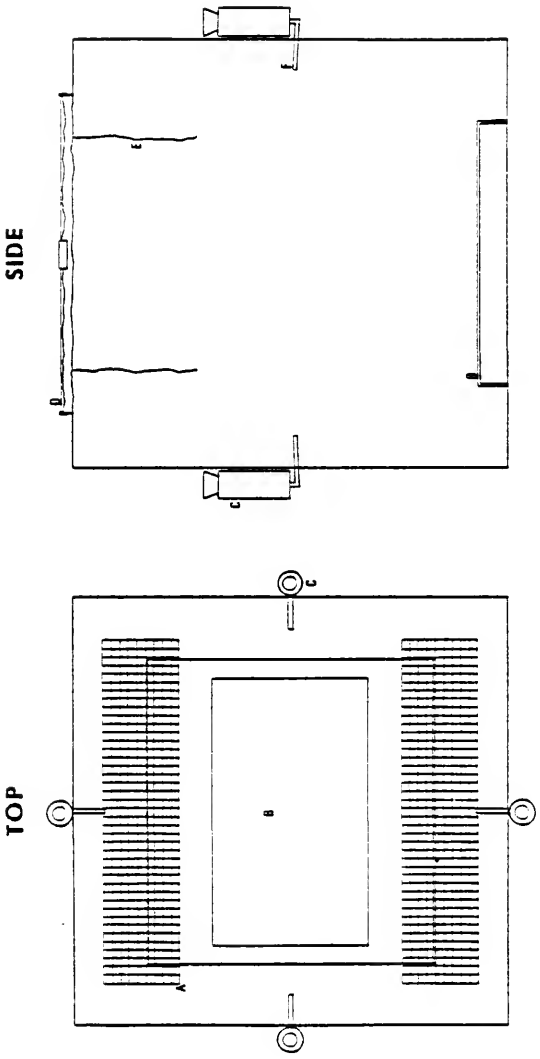

SIDE
TOP
A
B
C
D
E

Fig. 22. A 36.0 x 39.4 x 36.0-cm-high container for rearing fall armyworm larvae. A=cell unit filled with artificial diet; B=sterile vermiculite for pupation; C=aluminum angles to support cell units; D=threaded aluminum rods to assist late-instar larvae in crawling up to diet.

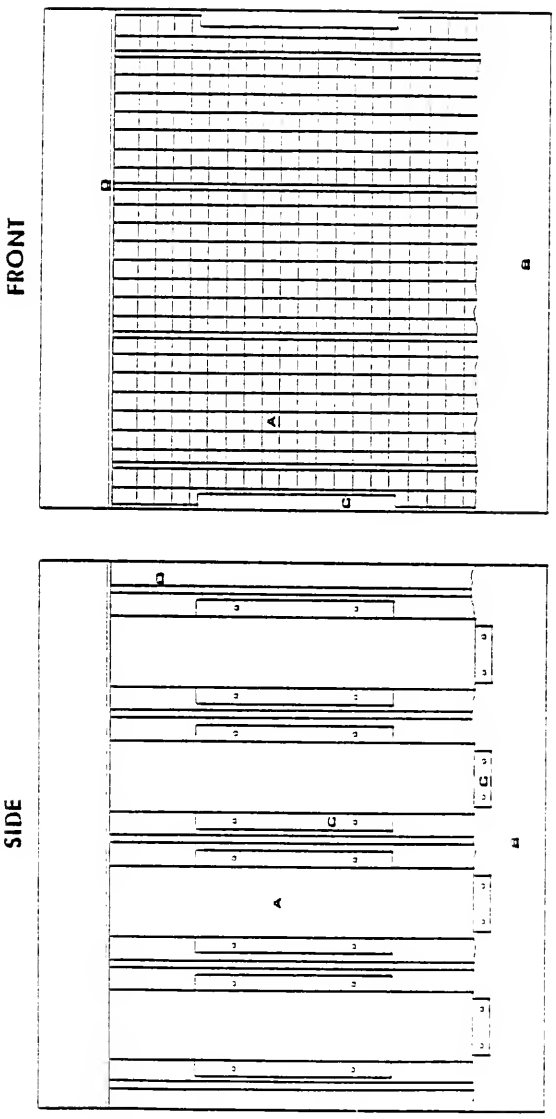

FRONT
SIDE

Fig. 23. Flow chart for identifying the causes of significantly reduced yields of usable fall armyworm pupae.

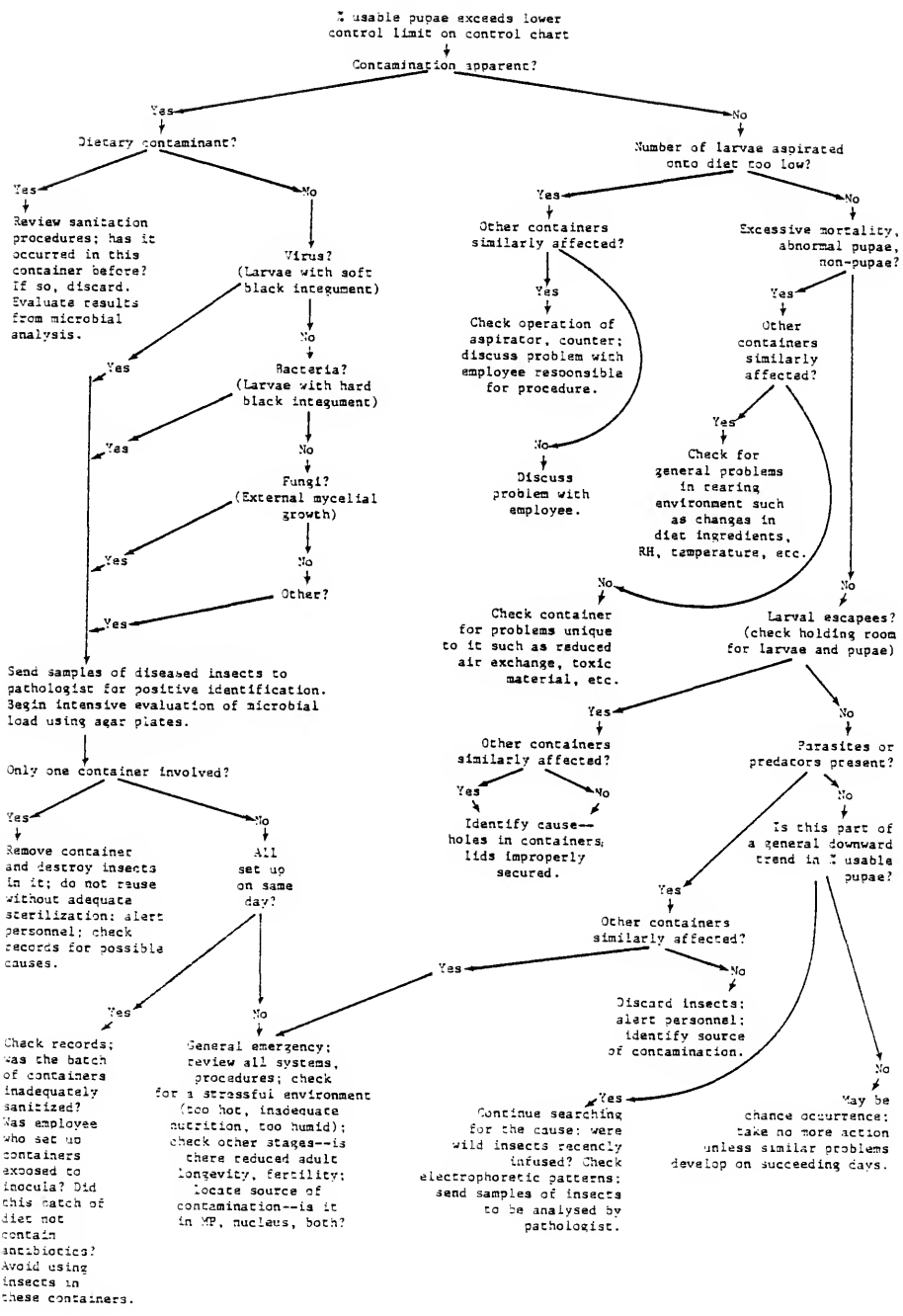
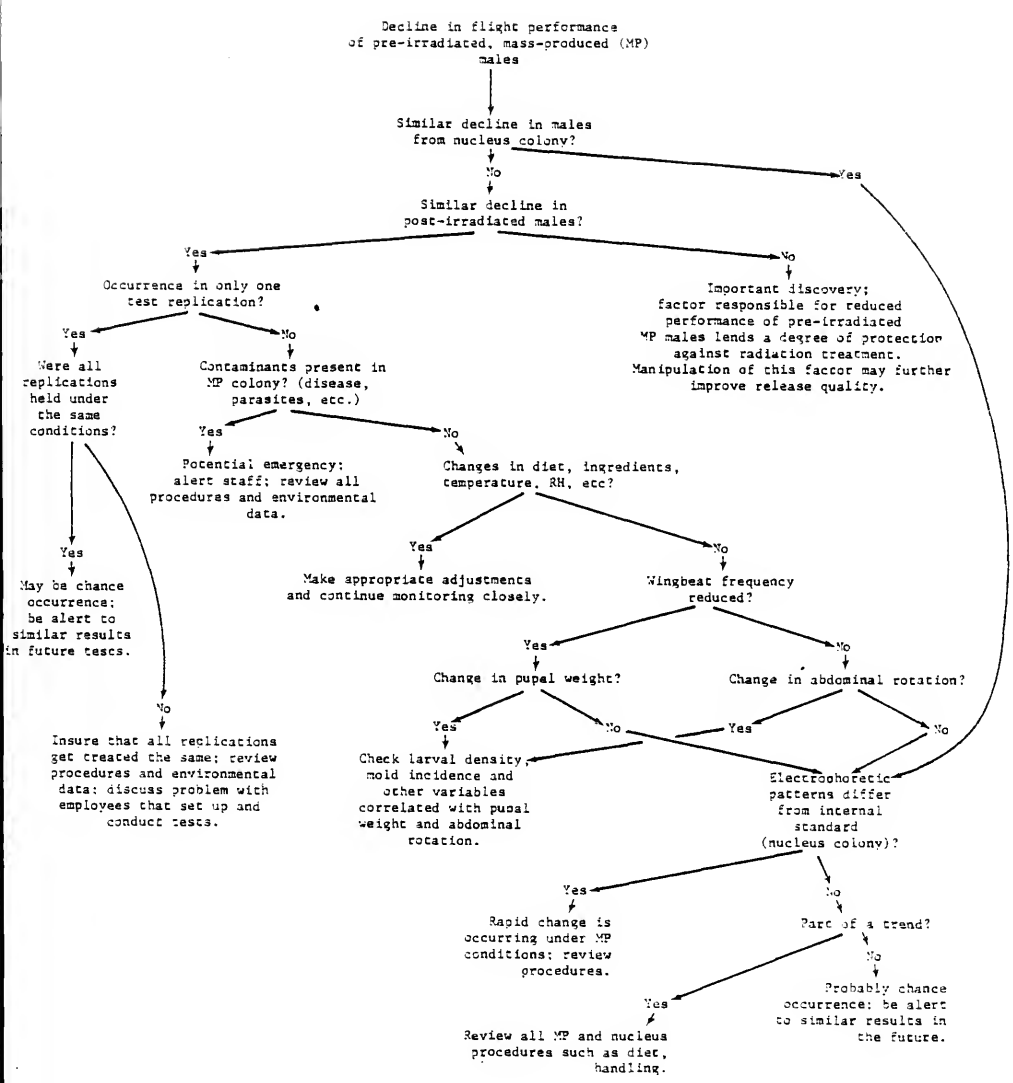

% usable pupae exceeds lower control limit on control chart
Contamination apparent?
Yes
No
Dietary contaminant?
Yes
No
Review sanitation procedures; has it occurred in this container before? If so, discard. Evaluate results from microbial analysis.
Virus? (Larvae with soft black integument)
Yes
No
Bacteria? (Larvae with hard black integument)
Yes
No
Fungi? (External mycelial growth)
Yes
No
Other?
Yes
Send samples of diseased insects to pathologist for positive identification. Begin intensive evaluation of microbial load using agar plates.
Only one container involved?
Yes
No
Remove container and destroy insects in it; do not reuse without adequate sterilization; alert personnel; check records for possible causes.
All set up on same day?
Yes
No
Check records; was the batch of containers inadequately sanitized? Was employee who set up containers exposed to inocula? Did this batch of diet not contain antibiotics? Avoid using insects in these containers.
General emergency; review all systems, procedures; check for a stressful environment (too hot, inadequate nutrition, too humid); check other stages--is there reduced adult longevity, fertility; locate source of contamination--is it in MP, nucleus, both?
Number of larvae aspirated onto diet too low?
Yes
No
Other containers similarly affected?
Yes
No
Check operation of aspirator, counter; discuss problem with employee responsible for procedure.
Discuss problem with employee.
Excessive mortality, abnormal pupae, non-pupae?
Yes
No
Other containers similarly affected?
Yes
No
Check for general problems in rearing environment such as changes in diet ingredients, RH, temperature, etc.
Check container for problems unique to it such as reduced air exchange, toxic material, etc.
Larval escapees? (check holding room for larvae and pupae)
Yes
No
Other containers similarly affected?
Yes
No
Identify cause--holes in containers, lids improperly secured.
Parasites or predators present?
Yes
No
Is this part of a general downward trend in % usable pupae?
Yes
No
Other containers similarly affected?
Yes
No
Discard insects; alert personnel; identify source of contamination.
Continue searching for the cause: were wild insects recently infused? Check electrophoretic patterns; send samples of insects to be analysed by pathologist.
May be chance occurrence; take no more action unless similar problems develop on succeeding days.

Fig. 24. Flow chart for identifying the causes for a decline in flight performance of mass-reared (MP) fall armyworm males.


Decline in flight performance of pre-irradiated, mass-produced (MP) males
Similar decline in males from nucleus colony?
No
Yes
Similar decline in post-irradiated males?
Yes
No
Occurrence in only one test replication?
Yes
No
Important discovery; factor responsible for reduced performance of pre-irradiated MP males lends a degree of protection against radiation treatment. Manipulation of this factor may further improve release quality.
Were all replications held under the same conditions?
Contaminants present in MP colony? (disease, parasites, etc.)
Yes
No
Yes
No
Potential emergency; alert staff; review all procedures and environmental data.
Changes in diet, ingredients, temperature, RH, etc?
Yes
No
May be chance occurrence; be alert to similar results in future tests.
Make appropriate adjustments and continue monitoring closely.
Wingbeat frequency reduced?
Yes
No
Insure that all replications get treated the same; review procedures and environmental data; discuss problem with employees that set up and conduct tests.
Change in pupal weight?
Change in abdominal rotation?
Yes
No
Yes
No
Check larval density, mold incidence and other variables correlated with pupal weight and abdominal rotation.
Electrophoretic patterns differ from internal standard (nucleus colony)?
Yes
No
Rapid change is occurring under MP conditions; review procedures.
Part of a trend?
Yes
No
Review all MP and nucleus procedures such as diet, handling.
Probably chance occurrence; be alert to similar results in the future.

CONCLUSIONS

Concepts of quality control are becoming established and accepted as a means to help ensure consistent production of insects that are able to function in their intended roles. Tests have been developed to measure many aspects of insect quality with modifications required for certain species and applications, and technological advances are improving their sensitivity and reliability. The next phase of quality control development will emphasize application in specific programs, as has been done with the fruit flies. Consequently, divergence in testing procedures, standards, and interpretations will occur among different groups of insects and result in very specific strategies. More importantly, quality control concepts will become comprehensively intregated into the entire production/release programs.

Entomologists realize that quality is based on the essential resources available to a program: insects, facilities, procedures, and personnel. No one resource can adequately compensate for deficiencies in the others. Hence, quality control strategies will be incorporated into facility design and program development so that important variables such as genetic changes and contamination can be

minimized. Personnel will be specifically trained to meet this challenge. Future research will provide the data for establishing more comprehensive tests and standards, for identifying the effects of program variables on the insects, and for managing the variables to improve their quality.

APPENDIX

Ingredients in one liter of the larval diet.

Ingredient	Amount
Primary Ingredients	
Casein	26.4 g
Pinto Beans	72.6 g
Torula Yeast	33.0 g
Wheat Germ	52.8 g
Gelling Agent	
H.W.G.*	12.2 g
Vitamins	
Ascorbic Acid	3.4 g
Vitamin solution	7.9 ml
Antimicrobials	
Methyl Paraben	2.1 g
Formaldehyde	4.0 ml
Sorbic Acid	1.1 g
Tetracycline	0.3 g
Water (deionized)	1.0 liter

* Carrageenan edible potassium-cloride.

Ingredients in vitamin mixture* for larval diets (Roche Chemical Custom Premix No. 15911 for USDA).

Vitamin	Amount/gram
Vitamin A	22,026.0 IU
Vitamin E	8.0 IU
Vitamin B12	2.0 g
Riboflavin	0.5 mg
Niacinamide	1.0 mg
d-Pantothenic Acid	0.9 mg
Choline	43.5 mg
Folic Acid	0.3 mg
Pyridoxine	0.2 mg
Thiamine	0.2 mg
d-Biotin	20.0 g
Inositol	20.0 mg

*Stock suspension consists of 400 g of this mixture per liter of deionized water.

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BIOGRAPHICAL SKETCH

William R. Fisher was born in Philadelphia, Pennsylvania, in September 1948. Shortly thereafter, his family moved to Southern California where he graduated from Lutheran High School in 1966 and California Lutheran College in 1970 with a B.A. degree in biological sciences. He then worked as a Senior Biological Technician in the Life Sciences Department at Stanford Research Institute (SRI) in Menlo Park, California. In 1976, Mr. Fisher received an M.S. degree in entomology from Mississippi State University after which he was employed as a Biologist by Dow Chemical U.S.A. in Walnut Creek, California. In 1979 he married Ms. Jeanne Wiegand and moved to Gainesville, Florida, where he became employed as a graduate research assistant supported by the U.S. Department of Agriculture during his doctoral program at the University of Florida.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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